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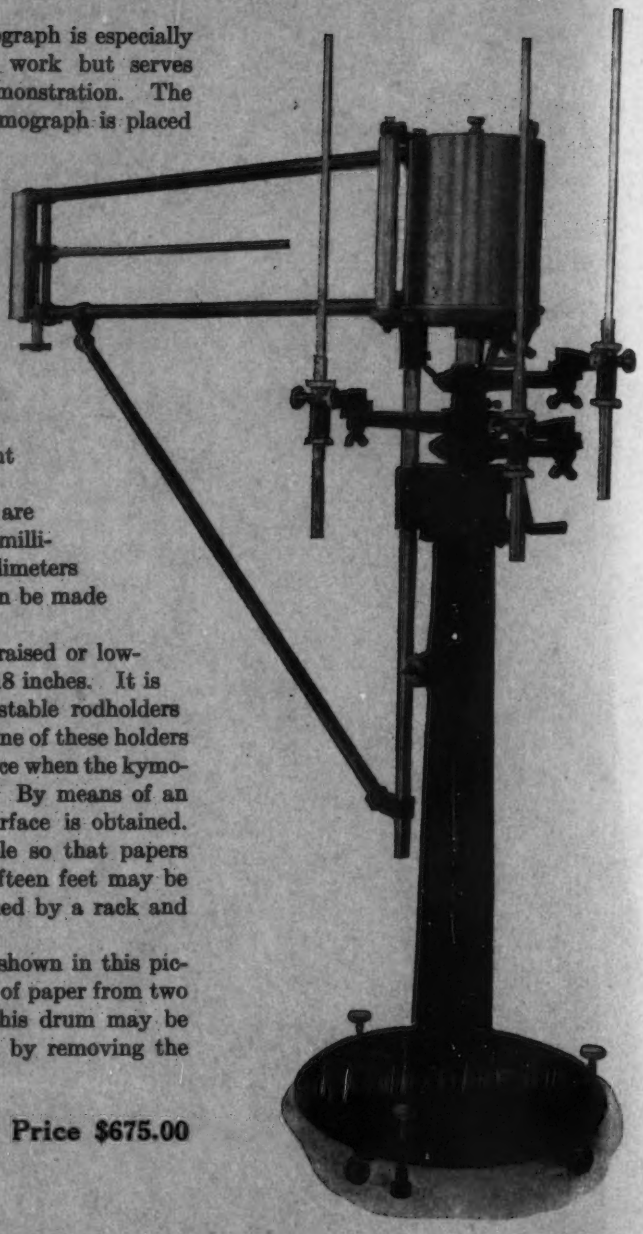

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THE INFLUENCE OF INSULIN ON THE MOTILITY OF THE URINARY BLADDER (Dog)

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From the Physiological Laboratories of the University of Chicago

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In 1924 Bulatao and Carlson reported an increase in gastric motility in normal fasting dogs as a result of injection of insulin and an inhibition of this activity with subsequent intravenous injection of dextrose. Quigley, Johnson and Solomon (1929) have noted a similar effect of insulin on the stomach of the normal fasting human subject. Quigley and Solomon (1930) extended their investigations to the human duodenum and to the dog's colon and in both cases reported that injection of insulin increased the motility of these organs. In view of these findings, which indicate that insulin has a more or less general and similar effect on the motility of the entire gastro-intestinal tract, it was decided to perform experiments on another smooth muscle viscus, the urinary bladder, as a step toward determining whether or not insulin causes an increase in activity of smooth muscle in general.

Methods. Normal unanesthetized female dogs were used throughout. The animals were trained to lie still on comfortable pads for periods of four hours. There was an average period of 16 hours between the last feeding and the start of the experiments. Insulin was injected subcutaneously in doses of 5 to 60 units, and dextrose was injected intravenously in 50 per cent solution. The usual experimental procedure was to take a control tracing of approximately 15 minutes' duration at the start of each experiment. Without interruption of the record insulin was injected, and the tracing was continued for about 4 hours. Control experiments lasting 4 hours were run at intervals on each dog.

Two methods of obtaining tracings of urinary bladder motility were tried. In the first a small condom balloon on a no. 10 hard rubber catheter was attached to a water manometer. The balloon and catheter were placed in 0.1 per cent $HgCl_2$ for several minutes and then washed with

sterile water. The vulva was swabbed with mercurochrome, and the balloon was inserted as aseptically as possible through the external urethral orifice into the bladder. The inflation pressure averaged 7.5 cm. of water.

The second method was similar in principle to that of Mosso and Pellacani (1882) in that a fluid system was used for recording volume changes. The fluid used consisted of one part of hexylresorcinol solution (1:1000) and three parts of 0.9 per cent NaCl. This fluid did not give rise to cystitis or urethritis, as determined at autopsy. The apparatus consisted of a calibrated pressure bottle connected with a manometer on the one hand and through a no. 14 hard rubber catheter with the interior of the urinary bladder on the other. The bulb on the pressure bottle was used to fill the system with fluid, after which a measured amount of fluid could be run into the urinary bladder. Depending on the size of the dog, the

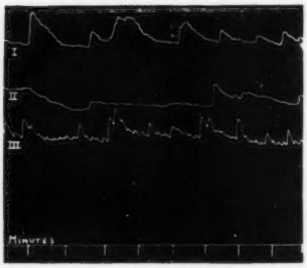

Fig. 1. Tracing of normal urinary bladder motility of 3 dogs by the fluid method, showing a maximum degree of normal motility.

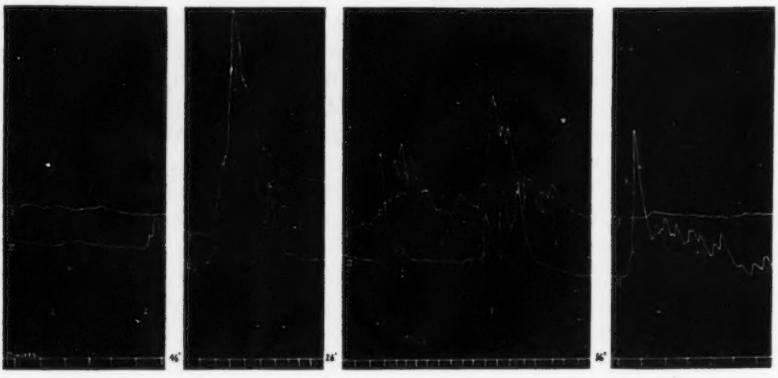

Fig. 2. Tracing showing effect of insulin on urinary bladder motility of 2 dogs by the fluid method. At *1* dog I was given 30 units of insulin subcutaneously. At *2* dog II was given 24 units of insulin. At *3* dog I was given 40 cc. of a 50 per cent dextrose solution intravenously.

quantity of fluid in the urinary bladder at the beginning of the experiments varied from 35 to 75 cc. with intra-vesical pressures ranging from 5 to 12 cm. of water.

Operations. Perineotomy was performed on each dog, the vulvar slit being extended dorsally, in order to expose the external urethral

orifice and thus facilitate the passage of the catheter. When the fluid method was used on a dog, a second operation was performed at the end of a long series of experiments. The purpose of this operation was to place a condom balloon attached to a gold-plated cannula in the abdominal cavity. This operation made it possible to take control tracings of intra-abdominal pressure simultaneous with tracings of urinary bladder motility on the unanesthetized dog. A low rectus incision was made aseptically under ether anesthesia. The sterile cannula with the balloon attached was put into the abdominal cavity through this incision, and the free end of the cannula was brought out through the ventral mid-line, as low as practicable, by way of a second small opening made just large enough to allow the cannula stem to pass through. A flange on the cannula was brought

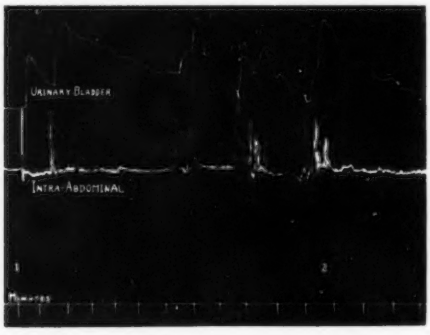

Fig. 3. Simultaneous tracings of urinary bladder motility and intra-abdominal pressure. The small excursions on the lower tracing are respiratory. There was hyperpnea during this period. *1*, 84 minutes after subcutaneous injection of 20 units of insulin. *2*, 15 grams dextrose intravenously.

to rest against the peritoneal surface. The omentum was drawn caudally so that it rested between the balloon and the intestines. The incision was closed and the animal was allowed to recover. The flange against the peritoneal surface kept the cannula and balloon from being pulled out. For tracings the extra-abdominal end of the cannula was connected with a water manometer and the intra-abdominal balloon was inflated. It was found that 5 to 6 days after the operation adhesions growing around the intra-abdominal balloon interfered with its inflation. Therefore control experiments on intra-abdominal pressure were run only at the end of a series of experiments.

RESULTS. *By the balloon method.* The balloon method was used in 28 experiments, including controls, on 3 dogs. Judgment of the degree of motility was based on the frequency and height of the contractions. A

rise in tone was considered to have occurred when the writing-point rose from the normal level and did not return within 10 minutes. According to these standards the different control tracings showed varying degrees of motility without any appreciable spontaneous increase in motility or tone. After insulin an appreciable increase in tone as well as in motility was observed in several experiments. The increase in urinary bladder motility following insulin usually began within an hour after injection

TABLE 1

Motility and tonus of the urinary bladder of the dog by the balloon method

The number of experiments falling in each of the four categories of degree of motility and tone is given by figures in the respective columns.

DEGREE OF ACTIVITY	15 CONTROL EXPERIMENTS		13 INSULIN INJECTIONS, 5–30 UNITS*	
	Motility	Tone	Motility	Tone
+	12	11	4	8
++	3	4	6	3
+++	0	0	3	2
++++	0	0	0	0

* Usual dosage 20 units.
\+ normal.
++ slight increase.
+++ moderate increase.
++++ marked increase.

TABLE 2

Motility and tonus of the urinary bladder of the dog by the fluid method

For explanation see table 1.

DEGREE OF ACTIVITY	90 CONTROL EXPERIMENTS		90 INSULIN INJECTIONS, 20–60 UNITS	
	Motility	Tone	Motility	Tone
+	64	73	7	11
++	22	14	14	9
+++	3	1	22	22
++++	1	2	47	48

and persisted until the end of the experiment. The rise in tone usually occurred about 2 hours after injection and persisted until the end of the experiment. Table 1 compares the results of insulin injections and controls.

By the fluid method. A total of 180 experiments half of which were controls and half experiments in which insulin was injected were done on 9 dogs. Periods of marked increase in motility or tone of the urinary bladder during the course of the control experiments were only rarely

observed. When insulin was given, however, there usually followed a marked increase in motility and rise in tone. The recorded changes after insulin, when the fluid method was used, were similar to but more marked than the changes recorded by the balloon method. Insulin convulsions, when they occurred, were always accompanied by micturition. In several experiments the activity of the bladder increased to such a degree that micturition occurred unaccompanied by convulsions or struggling. Since micturition usually necessitated terminating an experiment, an attempt was made to avoid convulsions by regulating the dose of insulin. In this series of 9 dogs the dose ranged from 20 to 60 units.

TABLE 3

Effect of dextrose on motility and tonus of the urinary bladder following insulin injection

The number of experiments falling in each of the six categories of results is given by figures in the respective columns.

RESULT	52 INTRAVENOUS INJECTIONS OF DEXTROSE	
	Motility	Tone
No effect	5	5
−	2	3
−−	5	6
−−−	10	10
−−−−	20	18
+++++	10 (?)	10

− slight diminution in activity.
−− moderate diminution in activity.
−−− marked diminution in activity.
−−−− return to normal.
+++++ further increase in activity over that following insulin.
(?) The increased activity following dextrose injection seemed to consist of a rise in tone although an increase in motility could not definitely be ruled out.

In none of the experiments in which intra-abdominal pressure was recorded was there any direct relationship between intra-abdominal pressure and the recorded motility of the urinary bladder. In two dogs the intra-abdominal balloons registered slight decreases in pressure more or less synchronous with bladder contractions. This might be expected since when the urinary bladder contracts and forces fluid out, the content of the abdominal cavity is diminished. On the other hand, such a fall in intra-abdominal pressure may have been adventitious, due to changes imposed on the balloon by intestinal motility synchronous with changes in bladder motility.

Effect of dextrose injection following insulin. In 52 experiments 10 to 15 grams of dextrose in 50 per cent solution were injected intravenously

when the urinary bladder activity was prolonged and at its height following insulin. The results of dextrose administration were variable. In the earlier experiments dextrose seemed to inhibit contractions consistently and to cause a decrease in tone to normal. There then usually followed a quiescent period lasting from 15 minutes to an hour, hyperactivity usually returning if the experiment was continued a sufficient length of time. Later experiments did not show such a marked and consistent effect of injection of dextrose. The impression was gathered, however, that individual contractions tended to be inhibited more than tone. In those experiments in which there was a balloon in the abdominal cavity, dextrose injection frequently brought about a rise in urinary bladder tone over that already present following insulin. The mechanism of the rise was not clear. Evidence of ascites was noted in some of these experiments, the abdominal wall being distended. The tracings of intra-abdominal pressure, however, showed no increases corresponding to the increases in bladder tone. This indicates that the mechanism was not a rise in intra-abdominal pressure due to the ascites. It is possible that the injection of dextrose intravenously in some instances caused a rapid diuresis which led to the rise in tone.

Discussion. Although the statement is frequently made that the normal motility of the dog's urinary bladder is very slight, 7 of the 9 dogs used here showed detectable motility normally. Elliott (1907) makes the generalization that "automatic rhythmic contractions practically do not occur in other bladders than those which possess inhibitory nerves." Yet Mosso and Pellacanis' (1882) tracings show that some dogs have considerable rhythmic activity. In this work normal rhythmic contractions seemed to be the rule rather than the exception.

The site of action of the insulin was not determined. Future experiments on the partially and totally denervated bladder may indicate the place of action of insulin in increasing the activity of the urinary bladder.

SUMMARY

1. Subcutaneous injection of insulin causes an increase in motility and tone of the unanesthetized female dog's urinary bladder.

2. Micturition with or without convulsions may occur at the height of the hyperactivity following insulin.

3. The recorded increases in bladder activity were not due to changes in intra-abdominal pressure.

4. The effect of dextrose administered intravenously on insulin hyperactivity is not constant, but inhibition is frequently noted.

5. In this series of dogs the majority showed a considerable degree of urinary bladder motility, prior to injection of insulin.

The writer wishes to express his appreciation to Dr. A. J. Carlson for facilities freely offered and for helpful guidance in this work, and also to Dr. M. M. Kunde for advice and assistance in technical difficulties.

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RELATION OF THE HYPOPHYSIS AND OVARIES TO EXPERIMENTALLY-INDUCED UTERINE BLEEDING IN MONKEYS

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The cyclical changes of the uterus are associated with concomitant changes in the ovary, and the ovarian function depends largely upon the secretion of the anterior lobe of the hypophysis (Smith, 1926; Smith and Engle, 1927, and Zondek and Aschheim, 1927), but the definition of menstruation, which is one of the most manifest symptoms of the cyclical changes in the uterus of the human and other primates, is not clear. However, it is recognized that the bleeding from the endometrium is the most important factor of menstruation. Corner (1927) has found in macaques that menstruation may proceed, at least for a limited period, without ovulation or the formation of a corpus luteum. Edgar Allen (1927, 1928b), Maddux (1930), and Morrell and associates (1930) have shown that when the follicular hormone (oestrin, folliculin, amniotin, etc.) is injected into spayed macaques, bleeding from the uterus results after the cessation of injections. Edgar Allen (1928a) found that the transplantation of the anterior lobe of the hypophysis in non-castrated immature macaques caused considerable growth in the genital tract and marked growth of the Graafian follicles. This led him to suppose that the development of the genital tract and the effects on the sex characteristics were secondary to changes in the ovary, for, as noted by him, these changes are similar to those obtained in normal and castrated monkeys from injections of ovarian hormone. The finding of Smith and Engle (1927) that anterior lobe implants were ineffective in castrated rats also supported this supposition of E. Allen.

Hartman, Firor, and Geiling (1930) were able to cause bleeding from the uterus in macaques by injection of extracts of the anterior hypophysis. In a series of experiments, they found evidence that the extract of the anterior hypophysis caused bleeding in hypophysectomized macaques while follicular hormone did not. This evidence with others led them to

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postulate the presence of a special anterior lobe hormone as the cause of bleeding, distinctly different from the follicle-stimulating hormone of Smith and Engle and the luteinizing hormone of Evans and Simpson (1928).

The object of the present work is to find out whether or not extracts of the anterior lobe of the hypophysis can cause external bleeding from the uterus like that found during normal menstruation as stated by Hartman and co-workers, and also to investigate further the relation of the ovaries to the phenomenon in question.

The animals used in this experiment were eight immature rhesus monkeys, weighing from 2400 to 3400 grams, and two mature rhesus monkeys. The immature animals were estimated to be between three and four years of age and had not passed through the first menstruation. The sheep hypophysis extract used was kindly made for us from whole hypophyses by Parke, Davis & Co. (See Bugbee, 1931.) One cubic centimeter of the extract corresponds to 0.25 gram of the fresh tissue of the hypophysis.

The first two experiments involved simply the administration of hypophysis extract to normal immature monkeys.

Macaque 69, weight 2475 grams, immature. Beginning February 19, 1931, 36 cc. of the hypophyseal extract were injected intraperitoneally over a period of 8 days. Increased reddening of the sexual skin became noticeable on the third day of the injections and persisted until the fourth day after the last injection when it began to fade. The swelling of the vulva began on the sixth day. Uterine bleeding, as manifested by external vaginal bleeding, began 8 days after the last injection and continued for 6 days. Microscopic bleeding, however, was apparent for 2 days more.

Macaque 70, weight 2810 grams, immature. Thirty-six cubic centimeters of the hypophyseal extract were injected over a period of 8 days. The reddening and swelling of the vulva were almost the same as seen in no. 69. The external bleeding began 7 days after the last injection and continued for 5 days. For 2 days before that and 3 days afterward erythrocytes were seen in the lavage microscopically.

These two experiments show that the injection of hypophyseal extracts can cause external bleeding which in amount and duration is similar to that found during normal menstruation. No gross bleeding was seen during the injections, but upon microscopic study of the lavage a few red blood cells were found on the second and third day. In my experience the number of red blood cells found at this time was no greater than those occasionally observed in normal untreated monkeys.

In the next three experiments injections were continued longer to see whether bleeding might occur during the injections.

Macaque 82, weight 3300 grams. Fifty-nine cubic centimeters of the extract were injected over a period of 17 days. Swelling of the vulva began on the fourth day and became very marked near the end of the period of injection, but no bleeding was seen. Typical bleeding began 6 days after discontinuing the injections and lasted for 7

days. However, microscopic bleeding was observed for 1 day before and 9 days after the external bleeding. The color of the sexual skin and the swelling of the vulva began to regress soon after the injections were discontinued, and 1 day before the onset of bleeding they had practically disappeared.

Macaque 60, weight 3390 grams (mature?). This animal had menstruated only once since she was purchased in May 1930, namely, in October 1930 for 3 days. Fifty-nine cubic centimeters of the extract were injected over a period of 17 days beginning January 26, 1931. There was no bleeding during the injection, except for a few red blood cells which were seen microscopically on the second and third days. In this case also the external bleeding occurred 8 days after discontinuing the injections and lasted for 7 days, a day before that and 3 days after that microscopically. The swelling and reddening were similar to those found in no. 82.

Macaque 72, weight 3150 grams, immature. Forty-three cubic centimeters of the extract were injected over a period of 14 days. The reddening and much swelling were observed. Six days after stopping the injections the typical external bleeding began, but no bleeding was seen microscopically or macroscopically during the injections.

In all cases, therefore, where injections were continued for 14 or 17 days, the menstruation-like bleeding occurred after discontinuing the injections, in exactly the same manner as when injections were made for only 8 days.

The next three experiments were designed to show whether or not the bleeding may be induced at intervals shorter than the normal menstrual cycle.

Macaque 82, second experiment. Three weeks after the onset of the last experimental bleeding 18 cc. of the extract were injected again over a period of 7 days. External bleeding was detected 5 days after the last administration and lasted for 6 days. Microscopical bleeding was observed for 2 days after this period, but neither during the injection nor before the external bleeding. Swelling and reddening were observed in this case also.

Macaque 69, second experiment. In no. 69, following the last day of the bleeding, 30 cc. of the extract were injected over a period of 7 days. The swelling and reddening began on the third day and external bleeding on the seventh day after the last injection, 3 weeks after the onset of the preceding bleeding. Microscopical bleeding was seen the day before and for 2 days after that. Seven days after this second experimental bleeding the animal was injected for the third time, 21 cc. of the extract being given over a period of 8 days. External bleeding began, for the third time, 9 days after stopping the injection. On the second day of this menstruation both ovaries and the uterus were removed surgically.

Macaque 70, second experiment. Two days after cessation of the first experimental bleeding this animal was again injected for a period of 7 days with 25.5 cc. of the extract. Swelling and reddening began during the period of injection. Bleeding appeared 5 days after the last injection and lasted for 6 days. Beginning 10 days later the animal was injected with 21 cc. of the extract in 8 days, but no bleeding was observed either macroscopically or microscopically. Very slight swelling for the last 3 days and a little reddening for the last 2 days of the injection were observed, however.

These experiments show that experimental menstruation-like bleeding can be produced 2 or 3 times in the same macaque and at intervals shorter than the normal cycle, by the injection of an extract of the hypophysis.

Four experiments were next performed in which the hypophysis extract was administered to spayed monkeys.

Macaque 65, mature. Menstruation had been irregular. Bilateral oöphorectomy was performed January 28. On January 30 some red blood cells were observed in the lavage and on January 31 a small blood clot was found in the vagina. After that there was no bleeding. On February 26 the injection of the hypophyseal extract was begun and continued until March 5, the doses totalling 51.5 cc. At various times, both during and after the period of injections, a few red blood cells were seen microscopically in the washings; for instance, 1 or 2 in some fields under high power. This slight and irregular bleeding, though detectable microscopically, was insignificant when compared with that produced in non-castrated animals by the same extracts. Two weeks later 34 cc. of the extract were injected in the animal over a period of 8 days, but no bleeding was observed either macroscopically or microscopically, and the animal died of an unknown cause 8 days after the last injection. During these injections no swelling of the vulva was seen.

Macaque 68, weight 3390 grams, immature. Oöphorectomy was performed March 16. Beginning April 10, 30 cc. of the hypophyseal extract were injected over a period of 11 days, but neither macroscopical nor microscopical bleeding was observed either during or after the injections.

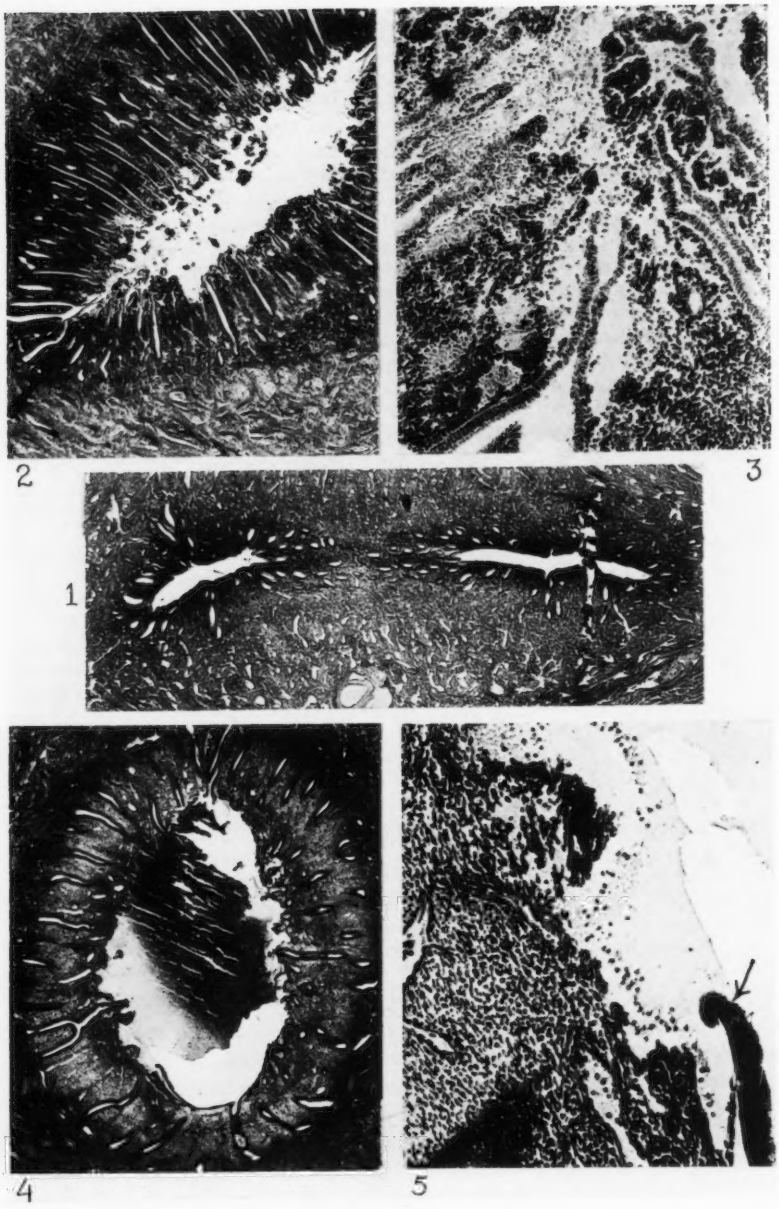
After this experiment this castrated macaque was used for other experiments, being injected with follicular hormone.

On September 17, when the animal weighed 3825 grams, the injection of the hypophyseal extract was begun again, amounting to 40 cc. in 9 days. No bleeding was seen either macroscopically or microscopically. Reddening and swelling were not detected during this period at all.

Macaque 93, weight 2470 grams, immature. After the castration, bleeding was seen because the extract of the hypophysis had been administered previously to produce it. Two weeks after the cessation of the external bleeding the injection of the hypophyseal extract was begun, amounting to 31 cc. in 8 days. Neither macroscopical nor microscopical bleeding was seen during or after the treatment, and no reddening or swelling was detected.

Macaque 49, weight 3825 grams, mature. This mature macaque had been menstruating regularly and was in good health. The last menstruation was observed for 5 days beginning August 24, 1931. On September 4, both ovaries were removed. Bleeding was detected again on September 9 and lasted for 5 days and microscopically for 2 days more. This must have been the result of the oöphorectomy, as noted by E. Allen (1926). On September 23 injection of the hypophyseal extract was begun, amounting to 40 cc. in 7 days. No bleeding was observed during or after the treatment. Neither swelling nor distinct increase of reddening was seen.

These experiments show, therefore, that injection of hypophyseal extracts into castrated monkeys caused no bleeding (6 cases using 4 macaques), whereas the same extract when injected into immature non-castrated monkeys did cause bleeding. It is logical to suppose then that this bleeding is dependent upon the presence of the ovary, and that the uterine changes follow the induction by the hypophyseal extract of some alteration in the ovary. Moreover, it must be added here that when bleeding was not produced by injections (the third experiment on no.



Figs. 1–5

70, non-castrated), swelling of the vulva was very slight when compared with that seen in the other cases.

THE UTERUS. The uterus was observed in several cases by exploratory laparotomy and in some instances was studied microscopically following hysterectomy.

Macaque 82. On the second day of the experimental bleeding a laparotomy was performed. The uterus was not notably larger than in normal immature monkeys of the same age.

Macaque 70. After the second series of injections, i.e., 2 weeks after the onset of the first experimental bleeding and 5 days before the onset of the second experimental bleeding, the uterus (observed by laparotomy) was as large as a mature uterus.

Macaque 69. Two weeks after the onset of the first bleeding and a week before the second bleeding, the uterus was as large as that of a mature animal. On the second day of the third experimental bleeding the uterus was removed and much blood was found in the cavity. Microscopical sections showed bleeding and desquamation from an interval stage of the endometrium (figs. 2, 3), just the same as in cases of normal spontaneous menstruation (without ovulation) described by Corner and E. Allen. It was found that the endometrium was thicker than normal, there was ragged mucosa with sloughing of superficial glands, and much blood. The glands were straight and not dilated, the gland epithelium was high, epithelial mitoses were absent and there were many lakes of extravasated blood in the subepithelial tissue

Macaque 72. On the second day of bleeding the uterus was removed and found to be enlarged and hyperemic and to contain much blood in the cavity. The histological examination showed almost the same findings as in no. 69 (figs. 4, 5). Moreover many subepithelial capillaries were seen to be much dilated and connected with lakes of extravasated blood.

THE OVARY. *Macaque 70.* After the series of injections 5 days before the experimental bleeding, the exploratory operation showed several small follicles at the surface of each ovary but no recent or old corpora lutea were seen.

Macaque 82. On the second day of the experimental bleeding the exploratory operation showed that the ovaries were rather small and had a few small follicles but no corpora lutea were visible.

Macaque 72. On the second day of the experimental bleeding, the ovaries were

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Fig. 1. Section of uterus of a normal immature macaque. Photograph, × 15. Hematoxylin and eosin. Endometrium is much thinner than in the pictures which follow.

Fig. 2. Section of endometrium, macaque 69. Photograph, × 15. Section shows desquamation of the epithelium and superficial portions of the glands. Blood in the cavity. Glands of interval type.

Fig. 3. Higher magnification (× 100) of the endometrium shown in figure 2. Ragged mucosa with sloughing of superficial glands. Blood in cavity and many lakes of extravasated blood in subepithelial zone.

Fig. 4. Section of uterus, macaque 72. × 15. Blood in cavity, desquamation of the epithelium. Glands of interval type.

Fig. 5. Higher magnification (× 100) of the endometrium shown in figure 4. Epithelium has disappeared or is being desquamated (see arrow). Dilated capillaries full of blood appear to be connected with lakes of blood.

removed with the uterus and examined microscopically. Neither large follicle near ovulation nor corpus luteum was found.

Macaque 69. In the interval between the two experimental bleedings the ovaries were found by exploratory laparotomy to be as large as those found in fully mature animals. Many small follicles were present but no large follicles nor corpora lutea were observed. On the second day of the third experimental bleeding the ovaries were removed. Microscopic sections showed many medium and small-sized follicles

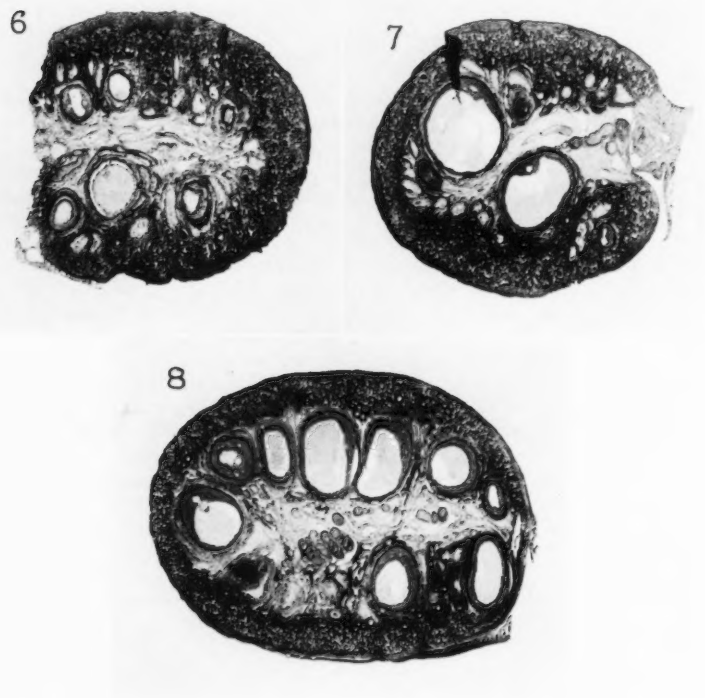

Fig. 6. Section of the right ovary, macaque 93, just before the second injection of the smallest amount of the hypophyseal extract sufficient to cause bleeding. Photograph, × 10.

Fig. 7. Section of the left ovary, macaque 93, just after the above mentioned injection (15 cc.). Follicles are distinctly larger than follicles of the right ovary (fig. 6), but otherwise not much different from the right. Photograph, × 10.

Fig. 8. Section of the right ovary, macaque 29, after three series of injection of the hypophyseal extract. Follicles are more in number and distinctly larger than in the ovary of another animal of the same size (fig. 6). Much vascularisation is to be seen. Neither corpus luteum nor large follicle near ovulation. Photograph, × 10.

in which liquor folliculi had been elaborated. There were 31 medium and 27 small follicles in the right and 26 medium and 39 small follicles in the left. They showed neither large follicles nor corpora lutea, consequently ovulation had not occurred. But when the ovaries of this animal are compared with those of the immature monkey of about the same age (fig. 6, 8) it is clearly seen that considerable follicular development had occurred.

A series of experiments, to be added here, will sustain the idea that the bleeding occurs when the stimulating effect of follicular hormone upon the endometrium is decreased after previously causing some changes in it.

In the first experiment of this series, it was attempted to elicit bleeding by injection of the follicular hormone into a castrated immature macaque.

Macaque 68, 3450 grams. This castrated immature macaque was first used for a control experiment, being injected with the extract of the hypophysis, but it was found impossible to produce bleeding in this way (see p. 11). Next, she was injected with 245 R. U. of follicular hormone (amniotin) over a period of 9 days. Reddening began on the fourth day (after the injection of 45 R. U.), and swelling began on the sixth day (after the administration of 75 R. U.). Nine days after the last injection the lavage had a reddish tinge, and on the following day much external bleeding began, which lasted for 6 days. Microscopical bleeding was seen for one day before and for 4 days after this period.

Seven weeks later 60 R. U. of the follicular hormone were given over a period of 5 days. Reddening began on the third day and swelling was seen on the fifth day. Thirteen days after the last injection the lavage showed a red tinge and contained many red cells. The next day external bleeding began and lasted for 4 days. Very few red cells were seen microscopically for 6 days before that and microscopical bleeding lasted 5 days after the external bleeding.

In this experiment, just as in those of E. Allen and Morrell, external bleeding was produced in a castrated immature macaque by injecting follicular hormone. The bleeding occurred several days after the cessation of the administration of the hormone. The doses used caused reddening of the sexual skin and swelling of the vulva and probably stimulated the endometrium too.

The fact that uterine bleeding could be produced by treatment with follicular hormone in this castrated immature animal demonstrates that failure to obtain the same result with hypophysis extracts in previous experiments on the same animal was not due to insensitiveness of the uterus.

In the next experiment an immature animal was given a small amount of hypophysis extract, insufficient to induce bleeding, but sufficient to cause some stimulation of the ovaries as evidenced by reddening of the sexual skin. The animal was then spayed.

Macaque 68, 3390 grams, immature. Before the castration, 13 cc. of hypophysis extract had been injected over a period of 4 days. This amount is thought to be insufficient to cause the external bleeding from the uterus (see the experiment on

macaque 93, p. 17). Slight reddening of the skin was seen on the fourth day, and the next day the oöphorectomy was performed. Seven days after that the external bleeding began and lasted for 5 days, and red blood cells were seen microscopically in the lavage for 3 days more.

Edgar Allen (1926, 1928b) caused experimental menstruation in adult monkeys by removal of both ovaries, but could not in immature monkeys. In my experiments even a mature monkey, which had menstruated only two times during the past 7 months, showed only a little bleeding on the third day after the removal of both ovaries (macaque 65). The immature macaque 68 had definite menstruation-like bleeding for 5 days, 7 days after the removal of both ovaries, but this may be explained by the previous administration of an insufficient amount of the hypophysis extract to cause menstruation-like bleeding by itself.

From these two experiments it is imaginable that the bleeding from the endometrium occurs when the stimulation of the follicular hormone is interrupted after it has caused certain changes in the endometrium. Also, the experimental bleeding a few days after cessation of the injection of the anterior hypophysis hormone can be explained in the same way, since it is clear that the hormone stimulates the ovary to produce follicular hormone.

If this supposition be true, then it follows that the bleeding which would normally be induced by the hypophysis extract should be postponed by administration of follicular hormone immediately after cessation of the hypophysis injections. This was tried in the following four experiments.

Macaque 70, immature. An amount of the hypophysis extract sufficient to cause the bleeding (30 cc.) was injected into this macaque over a period of 8 days, 6 weeks after the last experiment. The animal began to show reddening of the skin on the third day and some swelling on the fourth day of the treatment. On the ninth day injection of the follicular hormone (amniotin) was begun, which amounted to 460 R. U. in 12 days. The reddening and the swelling increased very much during the treatment but the bleeding was not seen which usually occurs on the fourth to ninth day from the last administration of the hypophysis hormone. After 8 days from the cessation of the amniotin injection external bleeding began and lasted 5 days.

Three months after the last day of this bleeding the same experiment was repeated in this macaque, namely, injection of the hypophyseal extract was continued for 6 days (similar dosage) followed immediately by injections of amniotin for 12 days (180 R. U.). The increase of the reddening and the swelling was seen. During this period, however, no bleeding was detected and 6 days after the last injection of the follicular hormone external bleeding was seen and lasted for 6 days.

Macaque 82, immature. Thirty cubic centimeters of the hypophysis extract were given over a period of eight days. Next day the injection of amniotin was begun which amounted to 460 R. U. in 12 days. Reddening was observed on the third day, some swelling was seen on the fourth day and these sex characteristics increased very much during the period of the amniotin injection. Nevertheless, bleeding was not detected during the period of injections and it was 7 days after the last administration of amniotin that bleeding was seen externally, lasting 6 days.

Three months after this experiment injections of the hypophysis extract were continued for 6 days (similar dosage) succeeded by injections of 180 R. U. of amniotin for 12 days. No bleeding was seen during or after the injections until external bleeding was detected 5 days after the last injection of amniotin. This bleeding lasted for 6 days.

The results of the experiments on these immature macaques indicate strongly that the bleeding does not occur during the time the process caused by the follicular hormone is going on.

An additional experiment was undertaken to study the difference between the ovary of an immature macaque before and after the administration of the smallest amount of the hypophysis extract sufficient to cause the bleeding.

Macaque 93, 2470 grams, immature. Fifteen cubic centimeters of the hypophysis extract were injected intraperitoneally over a period of 5 days. On the fourth and fifth day the reddening and on the fifth day the swelling was noticed. On the third, fourth and fifth day after the beginning of the injections, and on the fourth and fifth day after the last injection from 3 to 7 red cells were seen microscopically in each field of the high power, the presence of which I can not explain in any way. However, on the tenth day from the last injection the lavage was distinctly red and showed innumerable red cells microscopically and this condition continued for 6 days, but the external bleeding was hardly noticed by observation of the vulva.

In the previous report it was noted that 21 cc. of the extract could cause bleeding in one case but not in another case. In addition to these, macaque 93 showed distinct bleeding, but not so much as usually obtained, by the injection of 15 cc. It is easily supposed that the amount of 15 cc. to 20 cc. of this extract (1 cc. = 0.25 gram of fresh tissue) is the smallest amount able to cause the menstruation-like bleeding in an immature monkey of this size.

(The same macaque). Ten days after the bleeding the right ovary was taken out, to be used for histological control, and 2 days later the injection of the hypophysis extract was begun and a total amount of 15 cc. was given over a period of 6 days. Reddening began on the third day and swelling on the fifth day. The day after the last injection the left ovary was removed. The lavage appeared distinctly red 9 days after the last injection and the same state lasted for 6 days; on the third of those days much external bleeding was detected. Microscopical bleeding followed for 5 days after the external bleeding and only a few red cells were seen microscopically for 4 days before that.

In the above mentioned experiments no corpus luteum or large follicle was found in either ovary. By microscopical examination the largest follicles in the left ovary removed at the end of the course of injections (fig. 7) seem to be twice as large as those of the other one (fig. 6), but no other remarkable difference was noted

Ovaries of an immature macaque (no. 65, p. 11) which had been caused

to bleed three times at rather short intervals by the administration of the hypophysis extract, showed many follicles larger than those of a normal immature macaque.

Since it is clear that the menstruation-like bleeding is closely correlated with the function of the ovary, much histological difference between those ovaries might have been expected; but the above mentioned data show rather that the small amount of follicular hormone sufficient to cause the bleeding and some other ovarian symptoms can be secreted from an ovary without any marked histological changes.

The mechanism of this experimental menstruation-like bleeding, produced by the injection of an extract of the hypophysis, remains to be discussed. It is unnecessary from these experiments to postulate a separate anterior lobe hormone as the direct cause of menstruation and much more logical to explain this menstruation-like bleeding as the result of stimulation of the ovaries by the anterior lobe hormone because: 1. The spayed monkeys did not bleed. 2. The menstruation-like bleeding did not occur during the injection of the extract but only several days after the treatment. 3. The bleeding was always preceded by the secondary sex characteristics of reddening and swelling of the sexual skin, and the uterus showed very clearly enlargement, congestion, and increased thickness of endometrium. All of these signs are known to be produced by the follicular hormone. 4. Though there were no large follicles near ovulation or corpora lutea in the ovaries at the time of the menstruation-like bleeding, some development of the ovaries was recognizable. 5. As shown by others and confirmed in these experiments, similar bleeding often occurs a few days after oöphorectomy, and is produced in the spayed monkey following the administration of follicular hormone. 6. The bleeding which invariably follows injection of the hypophysis extracts may be postponed by administration of follicular hormone.

On the basis of these experiments, it is tentatively assumed that the menstruation-like bleeding of the endometrium occurs after a certain physiological state of the endometrium caused by the follicular hormone is altered when the hormone is removed or reduced in amount. The effect of the hypophysis extract is, under this interpretation, simply to stimulate production of the follicular hormone, which is then reduced in amount after cessation of the hypophyseal injections. It seems to me to be more difficult to explain this bleeding as a result of the action of a special substance from the hypophysis.

SUMMARY

1. Menstruation-like bleeding from the uterus was caused by the injection into immature monkeys of an extract of the hypophysis (11 positive cases in 12). Intervals between the last injection and the external bleed-

ing were from 4 to 9 days, and the duration of the external bleeding was from 5 to 7 days.

2. This menstruation-like bleeding was produced 2 or 3 times at short intervals.

3. Castrated monkeys did not bleed microscopically or macroscopically during or after the injection of the anterior lobe extract

4. The ovaries of the monkeys in which bleeding was produced by the treatment showed no ripe follicles or corpora lutea but there was some difference in the ovaries, as shown by experiments in which the follicles were larger in the ovaries removed after the injections than in the control ovaries. This experimental menstruation-like bleeding was similar to that found in normal monkeys where regular menstruation may occur without rupture of a follicle or presence of a corpus luteum.

5. The uteri from the positive cases showed bleeding from an interval stage of the endometrium.

6. A castrated immature monkey, which did not bleed following injections of hypophysis extract, showed menstruation-like bleeding subsequently after a course of injections with follicular hormone.

7. An immature monkey treated with a sub-effective dose of hypophysis extract and then spayed, showed bleeding after removal of the ovaries.

8. Bleeding which normally follows treatment with hypophysis extract was postponed by injections of follicular hormone.

9. It is assumed from the above experiments that the experimental menstruation-like bleeding results from an alteration of the endometrium which occurs when the effect of follicular hormone is removed.

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A QUANTITATIVE STUDY OF PLACENTAL TRANSMISSION AND THE PERMEABILITY OF FETAL MEMBRANES AT VARIOUS STAGES OF PREGNANCY

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In recent observations on the placental transmission of certain hormones following injection of them into the fetus, Snyder and Hoskins (1928 a, b) obtained no evidence of changes in the maternal organism that could be attributed to escape across the placental boundary of the injected substances, namely, insulin, adrenalin, pituitrin, and parathyroid. Accordingly, phenolsulphonphthalein was added to the substances to be injected into the fetus, in order to get evidence that the injected material had reached the fetal circulation, which would be shown by recovery of phenolsulphonphthalein from the maternal urine.

It became evident at once in these injections of phenolsulphonphthalein together with endocrine extracts that the escape of phenolsulphonphthalein across the placental boundary and appearance in the maternal urine occurred at a rapid rate and in considerable amount. The maternal urine was deeply colored with the dye in less than half an hour. This confirmed, in the rabbit, the previous observations of Wislocki (1921), who showed that phenolsulphonphthalein injected into guinea pig fetuses in utero could be recovered in the maternal urine forty minutes following injection.

In view of the well-known usefulness of phenolsulphonphthalein in obtaining quantitative data on kidney function in the human by merely comparing the amount of dye recovered in the urine in a given time with the amount injected, it seemed likely that quantitative measurement of the excretory processes of the fetus might be obtained in a similar manner, by determination of the output of phenolsulphonphthalein in the maternal urine, following the injection of the dye into fetuses in utero.

The selection of animals at various stages of pregnancy permitted detection of any change in amount of excretion as gestation approaches term.

In a second series of animals in which phenolsulphonphthalein was injected into the gestation sac, the attempt was made to measure the permeability of the chorio-amniotic membranes beyond the placental site

and to gain an estimate of the efficiency of the barrier between the amniotic fluid and maternal blood stream at various stages of pregnancy.

METHOD AND MATERIAL. The present experiments may be considered in three main groups, as summarized in tables 1, 2, and 3.

In the first group, the output of phenolsulphonphthalein was measured under uniform conditions of technique. Only the age of the embryos was varied in this series of observations.

In the second group, uniform conditions were similarly maintained except that the amount of phenolsulphonphthalein injected per fetus was varied; and in one experiment the number of fetuses injected was increased.

TABLE 1

Output of phenolsulphonphthalein in maternal urine following injection of fetuses in utero with 3 mgm. (0.5 cc.) per fetus

STAGE OF PREGNANCY	RABBIT NUMBER	WEIGHT OF FETUS	NUMBER OF FETUSES INJECTED	PHTHALEIN INJECTED PER FETUS	PHTHALEIN INJECTED PER GRAM OF FETUS	OUTPUT OF PHENOLSULPHONPHTHALEIN						CONTROL (OUTPUT OF MATERNAL ANIMAL 2 DAYS FOLLOWING FETAL INJECTION)	
						Period 1, 0-3½ hour (per cent of total, 6 mgm.)	Period 2, 3½-6th hour (per cent of total, 6 mgm.)	Period 3, 6th-12th hour (per cent of total, 6 mgm.)		Total in 6 hours		Total in 6 hours (mgm. per gram fetal body weight)	Phthalein injected	Output of phthalein in 2½ hours
								1st 3 hours	2nd 3 hours					
days		*grams*		*mgm.*	*mgm.*					*per cent*	*mgm.*		*mgm.*	*per cent*
21½	9	8	2	3	0.38	19	13			32	1.9	0.12	6	83
22	19	8	2	3	0.38	17	15	14	14	32	1.9	0.12	6	84
22½	11	6	2	3	0.50	19	14			33	2.0	0.16	6	80
23	8	11	2	3	0.27	17	18			34	2.0	0.09	6	
24	13	17	2	3	0.18	11	11			22	1.3	0.04	6	93
26	3	20	2	3	0.15	9	6			15	0.9	0.02	6	78
28	4	27	2	3	0.11	8	6			14	0.8	0.01	6	
29	23	32	2	3	0.07	10	9	3	3	19	1.1	0.02	6	82
29½	6	(35)	2	3	0.09	8	5			13	0.8	0.01	6	95

In the third group, observations were made under conditions identical with the first group except that phthalein was injected into the gestation sac instead of into the embryo.

The procedure in a typical experiment was as follows. The animal was fastened to the operating table on its back. A soft rubber tube was passed into the stomach through which amytal was given slowly, using 0.1 gram per kilo body-weight made up to a total volume of 200 cc. The large amount of water promoted adequate diuresis. The abdomen and genital region of the animal were shaved. Collection of urine by catheter is usually diffi-

cult in the female rabbit owing to the situation of the urethral orifice internally at a distance of about 10 cm. from the external opening of the

TABLE 2

Output of phenolsulphonphthalein in maternal urine following injection of fetuses in utero with various amounts of dye

STAGE OF PREGNANCY	RABBIT NUMBER	WEIGHT OF FETUS	NUMBER OF FETUSES INJECTED	PHTHALEIN INJECTED PER FETUS	PHTHALEIN INJECTED PER GRAM OF FETUS	OUTPUT OF PHENOLSULPHONPHTHALEIN						CONTROL (OUTPUT OF MATERNAL ANIMAL 2 DAYS FOLLOWING FETAL INJECTION)	
						Period 1, 0-3½ hours (per cent of total injected)	Period 2, 3½-6th hour (per cent of total injected)	Period 3, 6th-12th hour (per cent of total injected)	Total in 6 hours		Total in 6 hours (mgm per gram of fetus)	Phthalein injected	Output of phthalein in 2½ hours
days		*grams*		*mgm.*	*mgm.*				*per cent*	*mgm.*		*mgm.*	*per cent*
22	16	7	4	1.5	0.21	8	13		21	1.3	0.05	6	85
29	14	35	2	18.0	0.51	10	9		19	6.8	0.09	6	81
29	15	45	2	18.0	0.40	12	8		20	7.2	0.08	6	85

TABLE 3

Output of phenolsulphonphthalein in the maternal urine following injection of the gestation sac with 3 mgm. (½ cc.) of dye per fetus

STAGE OF PREGNANCY	RABBIT NUMBER	WEIGHT OF FETUS	VOLUME OF FLUID OF GESTATION SAC	SURFACE AREA OF FETAL MEMBRANES (EST.)	PHTHALEIN INJECTED PER FETUS	NUMBER OF SACS INJECTED	OUTPUT OF PHENOLSULPHONPHTHALEIN					CONTROL (OUTPUT OF MATERNAL ANIMAL 2 DAYS FOLLOWING FETAL INJECTION)	
							Period 1, 0-3½ hours (per cent of total, 6 mgm.)	Period 2, 3½-6th hour (per cent of total, 6 mgm.)	Period 3, 6th-12th hour (per cent of total, 6 mgm.)	Total in 6 hours		Phthalein injected	Output of phthalein in 2½ hours
days		*grams*	*cc.*	*sq. cm.*	*mgm.*					*per cent*	*mgm.*	*mgm.*	*per cent*
21	10	7	3	20	3	2	4	8		12	0.7	6	80
21	270	7	3	20	3	2	4	7	27	11	0.7		
25	22	15	5	35	3	2	11	6	8	17	1.0	6	87
26	21	14	5	35	3	2	11	16	11	27	1.6	6	72
29	20	47	1	60	3	2	42	12	12	54	3.2	6	78
30	17	30	2	50	3	2	43	5		48	2.9	6	
30	18	35	2	40	3	2	40	11		51	3.1	6	90

urogenital tract. Accordingly a funnel emptying into a flask was fitted tightly against the perineum. A braided silk thread attached to the skin

of the vulva was carried through the funnel and then over the neck of the flask to the table where it was held under tension.

Within an hour after administration of amytal, when the animal was deeply anesthetized, a lower midline incision was made under aseptic precautions and the uterus was exposed. The fetus was palpated and grasped below the costal margin so that a fine gauge needle could be inserted in the region of the dorsal musculature, passing through the thin uterine wall and enveloping membranes. The barrel of the syringe was then fitted into the needle, thus limiting the possibility of leakage into the maternal tissues during insertion of the needle into the embryo. A cotton sponge also surrounded the base of the needle near the junction with the syringe in order to detect and prevent escape of phthalein at this point. Care was taken to handle the uterus as little as possible especially near the placental site, and to avoid disturbance of the circulation such as is inevitable if the uterus is delivered through the abdominal wound. After the twenty-ninth day of pregnancy, however, premature expulsion of the fetuses becomes increasingly likely as term is approached, despite the above precautions. The abdomen was closed rapidly in two layers with purse string silk suture.

Under the influence of amytal the animal remained quietly on its back throughout the period of observation which was usually six hours, although occasionally as long as twelve hours.

Phenolsulphonphthalein is obtained in 1 cc. ampules which, as made up by Hynson, Westcott and Dunning for clinical use in test of renal function, contain 6 mgm. phenolsulphonphthalein per cubic centimeter of solution. Each fetus received a dose of 3 mgm. or 0.5 cc. phthalein solution, and two fetuses were injected with a tuberculin syringe graduated in 0.01 cc.

The urine collected during $3\frac{2}{3}$ hours following the injection of the embryos was isolated from the specimen obtained during the subsequent $2\frac{1}{3}$ hour period. When the experiment was continued over a twelve-hour period, the urine of the final six hours was isolated. At the conclusion of each period of the experiment the abdomen was opened again and the bladder filled with saline by direct injection through a fine needle, and then emptied in order to wash out thoroughly any remaining dye from the urogenital tract. About 25 cc. of saline were used in this way at each washing.

At the termination of the experiment the uterus was opened and the fetuses examined to see whether they were alive, as determined by beating of the heart. No experiments were included in which the death of a fetus occurred. The injected fetuses were then weighed and the average weight per fetus was recorded in the table. After emptying the uterus and removing the placentas, which could be accomplished with little loss of blood, the abdominal incision was sutured.

The quantity of phthalein recovered in the urine was then determined

colorimetrically. A convenient standard was made by diluting 0.5 cc. or 3 mgm. of the dye to a volume of one liter with distilled water and adding a few cubic centimeters of 25 per cent KOH until the color was fully developed. The urine containing an unknown amount of dye was then similarly made alkaline until the color no longer deepened and was diluted with water until the color matched that of the standard. Having determined the volume of the unknown urine specimen at the match point with the standard, the amount of phthalein was readily calculated. Error in colorimetric comparison arising from the pigments of the urine was eliminated almost entirely by this process of dilution as well as by the watery nature of the urine following diuresis resulting from the water intake of 200 cc.

About two days later the animal was injected intramuscularly with 6 mgm. of phthalein under amytal anesthesia as previously, and the output of phthalein in the urine during 2½ hours was determined. Thus the ability of the maternal animal to excrete phthalein in the urine was controlled by direct observation.

The stage of pregnancy could be accurately estimated since the animals were mated in the laboratory. Ovulation regularly occurs in this species about ten hours following copulation. The gestation period is about thirty-two days. The rabbits were reared in the laboratory on a liberal diet of oats, alfalfa, cabbage, salt and water.

Observations. *Group I.* In the first series of animals, as summarized in table 1, it is evident that during the last third of pregnancy there was a striking change in the output of phenolsulphonphthalein in the maternal urine following injection of fetuses in utero. When two fetuses of a litter were injected with 3 mgm. or 0.5 cc. phthalein per fetus, it was found that at the twenty-second day of pregnancy about 32 per cent of the phthalein was recovered within a period of six hours following injection, while near term, at twenty-nine days, only about 13 per cent was obtained. At an intermediate stage, in fetuses injected on the twenty-fourth day of pregnancy, it was found that the amount of phthalein recovered was likewise intermediate between the two extremes, being about 22 per cent of the total amount injected. The amount of phthalein which escapes across the placental boundary into the maternal blood stream is obviously much greater early in pregnancy than near term.

In control observations on the excretion of phthalein from the maternal organism following intramuscular injection of 6 mgm., at least 80 per cent of the dye was recovered in the urine within 2½ hours, indicating that the maternal kidney function was normal.

Additional details of the experiments, as recorded in table 1, are illustrated in figure 1. It is pointed out that the rate of excretion of phthalein tends to be more rapid early in pregnancy in both the first period of 3⅔

hours after injection and the second period of $2\frac{1}{3}$ hours. Thus in the experiment with no. 19, which was injected on the twenty-second day of pregnancy, the output in the first period was 17 per cent of the amount of phthalein injected, and in the second period was 15 per cent. On the other hand, in no. 6, which was injected on the twenty-ninth day, the output in the first period was about 8 per cent, while in the second period it was only 5 per cent.

In one animal, no. 19, observed on the twenty-second day of pregnancy, in which measurement of the phthalein output was extended over an additional six-hour period, making a total of twelve hours, about 60 per cent of

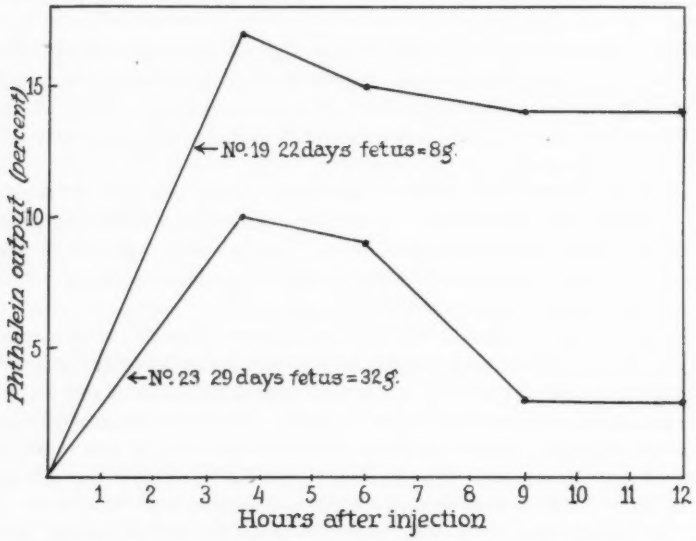

Fig. 1. Output of phenolsulphonphthalein in the maternal urine following injection of the foetuses at the 22d and 29th day of pregnancy respectively.

the dye was recovered. Since the output during the final six hours equalled 28 per cent of the amount injected, or about 14 per cent in each of two periods of three hours, there is evidence that the excretion of phthalein is maintained with little diminution during this period as indicated in figure 1.

In addition to this correlation of the response of the excretory mechanism of the fetal organism following injection of a constant amount of phthalein per fetus, with the age of the embryos, it is interesting also to take into account the change in weight of the fetuses, table 1. Since the weight of a fetus at twenty-two days averages about 8 grams while in a litter observed at twenty-nine days the weight is 35 grams, it is evident that when 3 mgm.

of phthalein are injected into each fetus, the dose per gram body-weight of the embryo is about 0.38 mgm. in the smaller fetus as compared with 0.09 mgm. in the larger, or four times as great. It is found that the smaller fetus excretes more than twice as much phthalein in six hours as the larger, the output being about 1 mgm. from the smaller compared with less than $\frac{1}{2}$ mgm. from the larger embryo. If the output per gram body-weight is considered, the difference between the smaller and larger fetus is striking. The fetus weighing 8 grams eliminates twelve times as much phthalein per gram body-weight as the fetus weighing 35 grams, or about 0.12 mgm. phthalein per gram body-weight in the former compared with 0.01 mgm. in the latter, during the period of six hours.

Group II. The next question, namely, the influence of increasing or decreasing the amount of phthalein injected, is considered in the experiments summarized in table 2.

In experiments with no. 14 and no. 15 the amount of phthalein injected into fetuses on the twenty-ninth day of pregnancy was increased in an attempt to equal the dosage per gram fetal body-weight which was injected into the smaller embryos of the twenty-second day of pregnancy. Thus in no. 15 with fetuses weighing 45 grams the amount of phthalein was increased six-fold, each fetus receiving 18 mgm. or 3 cc., thus giving a dosage of 0.40 mgm. phthalein per gram fetal body-weight. This closely parallels the amount given per gram body-weight in no. 19 with fetuses weighing 8 grams. With this increased dosage, the output of no. 15 was found to be about 20 per cent of the amount of phthalein injected, in contrast to 32 per cent in no. 19 with 8 gram embryos which received the same dosage of phthalein per gram body-weight. If the output per gram fetus body-weight is considered, it is found likewise that the output of the larger fetus is less than the smaller, being 0.08 mgm. per gram body-weight in the 45 gram fetus in contrast to 0.12 mgm. in the 8 gram fetus.

In the experiment with no. 16, having embryos of the twenty-second day of pregnancy weighing 7 grams, the injection of 6 mgm. or 1 cc. phthalein was divided among four embryos, each receiving 1.5 mgm. or $\frac{1}{4}$ cc. As a result the total output of phthalein was decreased to 21 per cent in six hours instead of about 32 per cent output which was observed when only two fetuses were injected with 6 mgm. of phthalein, each receiving only 3 mgm. Likewise the excretion per gram fetal body-weight was lowered to less than half the amount found after two fetuses were injected, being about 0.05 mgm. per gram body-weight when four embryos were injected, in contrast to 0.12 mgm. when two fetuses were injected. It is seen also that a greater amount of phthalein was eliminated in the second period after injection in no. 16 than in the first $3\frac{2}{3}$ hours. About 8 per cent of the amount injected was recovered in the first period while 13 per cent was excreted in the second part of the total six hours.

It is interesting to note that at the end of the experiments, the bladders of the fetuses that were injected with phthalein contained the dye, although not in sufficient quantity to be considered in the calculations of output. By sampling the bladder urine with a needle and syringe it was found easy to identify among the litter the fetuses that had been injected with phthalein.

Group III. In the third group of experiments as outlined in table 3, phthalein was injected into the waters surrounding the embryo instead of

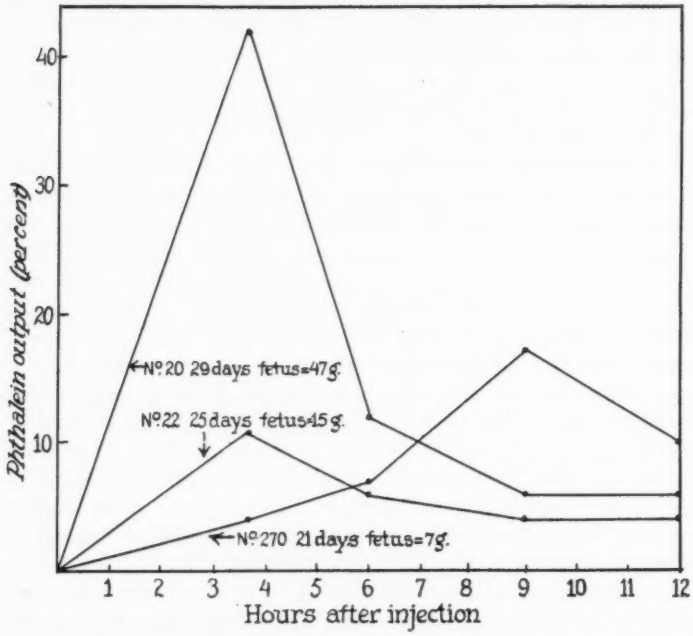

Fig. 2. Output of phenolsulphonphthalein in maternal urine following injection of the amniotic sac at the 21st, 25th, and 29th day of pregnancy.

into the fetal body. In each experiment the amniotic sac was injected in two fetuses, using 3 mgm. or ½ cc. phthalein in each instance, or a total injection of 6 mgm.

Observations under these uniform conditions gave striking evidence of changes in the output of phthalein from the gestation sac at various stages of pregnancy. In an animal, no. 10, observed on the twenty-first day of pregnancy, about 12 per cent of the phthalein escaped within six hours, while in an animal near term, no. 18, the thirtieth day of pregnancy, about 51 per cent of the dye was recovered, or four times as much. At an inter-

mediate stage, as in no. 22 (fig. 2) observed on the twenty-fifth day of pregnancy, only 17 per cent of the phthalein escaped into the maternal circulation in six hours. Indeed in this animal observation was extended over a period of twelve hours during which the total output was about 25 per cent, which was less than the amount excreted in the first period of $3\frac{2}{3}$ hours at the twenty-ninth day of pregnancy in no. 20.

If the output of phthalein is considered during the first period of $3\frac{2}{3}$ hours following injection, it is found that the excretion near term is about ten times greater than at the beginning of the last third of gestation. In an animal, no. 10, observed on the twenty-first day of pregnancy the output was 4 per cent in the first $3\frac{2}{3}$ hours while in no. 18, studied on the thirtieth day of pregnancy, about 40 per cent of the dye was recovered during this same interval. In the second period of collection, the excretion fell off markedly in no. 18, amounting to 11 per cent, while in no. 10 there was an increase to 8 per cent. During the entire six-hour period, therefore, the output is about four times as great near term as at the beginning of the last third of pregnancy. If the experiment is extended over a twelve-hour period, it is found that the output is maintained at a fairly low level during the final six hours, figure 2.

In connection with these findings there comes into consideration the change in surface area of the fetal membranes and the variation in the amount of fluid contained in the gestation sac with consequent change in dilution of the injected dye at various stages of pregnancy. In table 3 the volume of fluid is given as in the tabulation of Bruno Wolff (1909), which we also had opportunity to check in the course of the experiments. The surface area is calculated from the volume of the sac which is taken roughly to approximate the sum of the weight of the fetus plus the surrounding fluid. It is evident that as term is approached, the surface area of the fetal membranes is increased and the volume of fluid is diminished in amount so that there is less dilution of the injected dye at the surface of the limiting membrane. Both changes are in the direction of promoting increased elimination of phthalein, which of course supports the experimental findings. The magnitude of these physical changes, however, seems hardly adequate alone to account for the marked differences in output at various stages of pregnancy.

Discussion. In the foregoing observations, quantitative data have been obtained regarding the excretory processes of the fetus in utero. Although in the past countless substances have been injected into fetuses and into the amniotic sac in various species, this study is the first, as far as we know, that is based upon quantitative measurement of the excretory function.

The two anatomical channels along which products of fetal metabolism can conceivably reach the maternal blood stream have been studied in

separate series of animals. By aid of a dye, phenolsulphonphthalein, which is well known through its use in the study of human excretory function, and which is not toxic for the fetus, it has been possible to obtain a record, hour by hour, of the amount of the foreign substance which escapes by the two routes, that is, either across the placental boundary, or directly through the fetal membranes over an area less specialized than the placental region.

By selection of fetuses at various stages during the last third of pregnancy, during which period the fetal body-weight undergoes a five-fold increase, it has been possible to discover changes in excretion which might easily remain inconspicuous under less favorable conditions of observation.

An attempt has been made to correlate the output of phthalein across the placental boundary with various factors which could be kept under control, such as age of the fetus, weight, number injected, dosage of the dye, and time interval. Similarly in observations on the output directly through the chorio-amniotic membranes, correlation has been considered with regard to such factors as the surface area of the sac and the influence of dilution of the injected dye by fluid of the sac upon the final concentration at the surface of the limiting membrane. However, in explanation of the decrease in placental transmission of phthalein as term is approached and the increase in permeability of the fetal membranes to the dye, it is difficult to escape the conclusion that these changes represent in a certain measure a physiological parallel to the histological evidence (Mossman, 1926) of senility of the placenta and membranes as parturition draws near.

SUMMARY

1. In a study of placental transmission in a series of rabbits observed during the last third of gestation, it was found that following the injection of fetuses in utero with 3 mgm. of phenolsulphonphthalein per fetus there is an output during a six-hour period of more than 30 per cent of the dye from the fetus at the twenty-second day of pregnancy, while less than 15 per cent of the dye is excreted from the fetus at the twenty-ninth day.

2. If allowance is made for a sixfold increase in weight of the embryos near term, and the amount of phenolsulphonphthalein injected per fetus is increased to 18 mgm., there is still an output during a six-hour period of only 20 per cent of the dye from the fetus at the twenty-ninth day.

3. In a study of the permeability of fetal membranes in a series of rabbits observed during the last third of gestation, it was found that following the injection of the gestation sac with 3 mgm. of phenolsulphonphthalein per fetus there is an output during a $3\frac{2}{3}$ hour period of about 4 per cent of the dye from the sac at the twenty-first day of pregnancy, while about 40 per cent of the dye is excreted from the sac at the thirtieth day.

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RHYTHMIC VASCULAR UTERINE CHANGES[1]

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Although a great deal of work has been done on the changes in the uterus during the sex cycle in mammals, very few observations have been made on its rhythmic vascular changes. As reported in 1929, the color of the uterus of the guinea pig undergoes rhythmic variations that make the mucous membrane appear to blush and blanch every 15 to 20 seconds. These vascular changes are influenced by the time of day; being slowest and least intense in the early morning, increasing both in speed and intensity in the forenoon, decreasing again about noon and reaching their height of speed and intensity late in the evening. They are also influenced by the stage of the oestrous cycle, slowing during the pro-oestrum until they disappear completely for four or five hours and then reappearing before the end of the period of heat. The color changes are caused by variations in the amount of blood in the capillaries and arterioles due to rhythmic contractions of the latter. They appear to be specific for uterine tissue. Similar vascular changes were not observed in other tissues *in situ* nor in transplants onto the iris, or pancreas, islands of Langerhans, ductus deferens, heart muscle or liver. That vascular changes like those studied in the transplants also occurred in the uterus *in situ* was proven by observing them in anesthetized guinea pigs *in situ* and in unanesthetized guinea pigs through a uterine speculum.

Since these vascular changes are greatly affected by anesthesia and trauma to the uterus and surrounding tissues, a celluloid window 50 mm. square was placed in the lateral abdominal wall and the mucosa of an opened uterine horn observed through the window. By this method, the endometrium can be observed *in situ* without concomitant anesthesia or trauma and the vascular changes have the same rhythm as large endometrial transplants in the anterior chamber of the eye. The vascular changes observed through the window are modified by the time of day and the stage of the oestrous cycle, just as in the transplants. The only disadvantage of this method is that the inside of the window becomes covered with connective tissue in about six weeks. Since the vascular changes can

[1] A considerable amount of the material presented in this study has been summarized in an abstract (Markee, 1929).

be satisfactorily observed for at least 23 months in iridial transplants most of the observations on the vascular changes were made on iridial transplants and the other three methods were used only as checks on our results.

SPECIFICITY OF THE RHYTHMIC VASCULAR CHANGES FOR UTERINE TISSUE. As a further check on the specificity of the vascular changes for uterine tissue observations were made on other tissues in lightly anesthetized animals and more transplants of pancreas, islands of Langerhans, heart muscle and liver were made to the anterior chamber of the eye. Adrenal, thyroid and the mucosa of the bladder also were transplanted onto the iris and rather extensive observations were made on gastric fistulae and Pavlov pouches. The stomach resembles the uterus in musculature and mucosa and if these vascular changes were general in character, the mucous membrane of the stomach would seem a good place to observe them. Since no vascular changes were found that resemble those in the uterus, we feel justified in concluding that they are specific for uterine tissue.

RELATION OF THE GONADS TO THE VASCULAR CHANGES. A. *The ovary.* A further indication of the specificity of this phenomenon lies in the fact that it does not appear until after puberty. As was stated (Markee, 1929) it was not observed in immature guinea pigs. Therefore uterine autotransplants were made onto the iris of 12 immature animals to discover whether rhythmic vascular changes would appear at sexual maturity. The transplants were made when the animals were 21 days old. In nine of these animals they became vascularized within 12 to 36 hours but there were no rhythmic vascular changes. The animals went into heat for the first time from 92 to 107 days later. In each animal there was a marked growth in the transplant and an increase in its color during the week preceding the first oestrual period. The vascular rhythm was first observed in each of the nine animals during the three days preceding the first oestrual period. When each of these animals was operated on an extra piece of endometrium was removed and transplanted to the eye of her mother. Five of the transplants were successful and the rhythmic color changes appeared in the transplants in all five mothers from 7 to 10 days after transplantation.

The appearance of the vascular rhythm at sexual maturity and within so short a time in transplants from immature daughters to mothers seems to indicate that the uterus must be near or in a mature state before this phenomenon can manifest itself. The appearance of the vascular rhythm in so short a time in the daughter to mother transplants calls to mind the work of Zondek and Ascheim (1926) and P. E. Smith (1926) on the effect of transplants of the oral hypophysis upon the infantile uterus.

Since the color appeared at sexual maturity it seemed advisable to observe the effects of ovariectomy on the vascular rhythm in successful

transplants. Accordingly, 18 guinea pigs were ovariectomized in the middle of their dioestrous periods. For the first 5 days there was no perceptible change in the vascular rhythm. From that time on there was a gradual lengthening of the individual rhythms and a gradual decrease in the vascularity of the whole transplant until by the 45th day the vascular changes had nearly disappeared and the transplant had the appearance of immature endometrium. The time of disappearance of the vascular rhythm varied from 39 to 61 days.

An attempt to determine the effect of ovarian hormone[2] on these vascular changes was suggested both by the disappearance of the vascular phenomenon after ovariectomy and by the continuous blush of oestrus. Injection of the hormone, either subcutaneously or intravenously, into animals with intact ovaries slows the vascular rhythms and within 20 minutes stops them in vasodilatation. The length of time that the blush lasts is roughly proportional to the amount of hormone injected. About $\frac{1}{10}$th of the amount of hormone required to induce the vaginal changes in a spayed guinea pig will induce a period of continuous blush in the uterine transplants.

B. *The testes.* Because of the cumulative evidence that the difference between maleness and femaleness is a matter of a quantitative rather than a qualitative difference, uterine mucosa was transplanted to the eyes of male guinea pigs and rabbits. The transplants in the eyes of males react in all fundamental respects as they do in females. Castration in the male has the same effect on the vascular changes in such transplants, as ovariectomy in the female. After the injection of 20 mouse units of ovarial hormone, twice a day, for a period of a week, the vascular reactions reappear just as they do in the female. Injection of ovarial hormone into males with intact testicles causes a period of vasodilatation just as in the female. Since the same amount of ovarial hormone is required in both the male and female to produce the above changes, it seems that there is no antagonism between the internal secretion of the testicles and the ovarial hormone, at least not in so far as the induction of the period of persistent vasodilatation is concerned. The endometrial transplants in the eyes of males persisted without any alteration in the vascular rhythm for 23 months and microscopic examination did not reveal any degeneration in the mucosa at the end of that time. It therefore seems that a substance is elaborated somewhere in the tissues of noncastrated males that prevents the degeneration of the endometrium and that this substance is lacking in castrated males.

C. *The relation to ovulation.* The temporal relation in the guinea pig between the period of continuous blush and ovulation was studied in a

[2] The preparation used was the ovarial hormone menformon of Laqueuer (1927) obtained from the Marvel Pharmacal Co., New York.

series of 11 animals. One ovary and the corresponding Fallopian tube was removed. If ovulation had occurred the ova were collected from the tube by Corner's method, transferred to a slide and studied under an oil immersion lens. They were measured and the number of polar bodies recorded. If ovulation had not occurred, ovulation was induced by puncturing a folicle and exerting slight pressure on the ovary near the latter. The ovum and the follicular fluid that flowed out of the opening were transferred to a slide and studied. In this way it was found that from 6 to 10 hours elapse between the beginning of the period of continuous blush and ovulation. As seen by table 1 ovulation usually occurs

TABLE 1

The relation of blush to ovulation

GUINEA PIG NUMBER	OVARY	TIME BETWEEN BEGINNING OF BLUSH AND EXAMINATION	TIME BETWEEN END OF BLUSH AND EXAMINATION	OVULATED	STAGE OF MATURATION
		hours	*hours*		
182	Right	6	2	No	Germinal vesicle intact
	Left	8	3	Yes	2nd spindle, 1 polar body
143	Right	7	3	Yes	2nd spindle, 1 polar body
196	Right	6	2	Yes	2nd spindle, 1 polar body
173	Right	5	1	No	Germinal vesicle intact
147	Right	4	0	No	Germinal vesicle intact
	Left	10	5	No	Germinal vesicle intact
157	Right	8	4	Yes	2nd spindle, 1 polar body
	Left	7	2	Yes	2nd spindle, 1 polar body
138	Right	8	4	Yes	2nd spindle, 1 polar body
	Left	6	2	No	Germinal vesicle intact
139	Right	9	4	Yes	2nd spindle, 1 polar body
	Left	7	2	No	Germinal vesicle intact
168	Left	8	4	Yes	2nd spindle, 1 polar body
192	Right	7	3	No	1st spindle
95	Right	7	3	No	No vesicle; spindle not observed

about one hour after the end of the period of blush but in one case it had not even begun five hours after the end of the period of blush. At present all that can be said of this time relation is that the period of continuous blush occurs during the rapid growth of the follicle, at the time of the formation of Robinson's (1918) secondary follicular liquor.

MULTIPLE TRANSPLANTS. As a further test of the specificity of the vascular rhythm, two or more pieces of endometrium were transplanted to the anterior chamber of the same eyes in 253 animals (26 guinea pigs and 227 rabbits). Different transplants in the same eye have rhythms of different lengths. For example, one rabbit had four successful transplants,

two in each eye. Each of the four transplants had an independent rhythm. During a given time (8 minutes) one of the transplants blanched 5 times, second 5, third 7, and the fourth 8 times. Because of this difference in rhythm two transplants will occasionally blanch simultaneously. The frequency of this occurrence, of course, depends on the relative lengths of the rhythms in the different transplants. However, if there is physical continuity between two transplants they have a common rhythm just as the entire uterus *in situ* has a common rhythm.

The rhythm, in the multiple transplants, should be more uniform in length if it were due to a change in the vascular system as a whole. It is, therefore, impossible to explain the vascular cycle in endometrial transplants on the basis of any general rhythmic change in blood pressure or any general rhythmic nervous discharge. Further under these conditions, neither changes in intraocular pressure nor changes in the iris can be offered as the cause of the changes in color.

In order to discover the possible presence of a nervous control of this phenomenon the cervical sympathetic chain was sectioned and stimulated. Although the usual changes were elicited in the pupil and the blood vessels of the ear, the vascular rhythm was not modified by either sectioning or stimulating the cervical sympathetic chain. Further evidence that this phenomenon is not under the direct control of the nervous system is furnished by the fact that the rhythmic vascular changes have been observed and demonstrated as soon as 20 hours after transplantation, and it is highly improbable that a nervous control could have been established within so short a time.

The rhythmic nature of these vascular changes calls to mind the rhythmic contractions of uterine muscle described by Keye (1923). That they are not due to passive compression of the blood vessels by the contraction of the uterine muscle is conclusively proven by transplanting pieces of pure uterine mucosa. However, there probably is some causal connection between the vascular changes and the muscular rhythm, for during oestrus, when the uterus is most congested, the uterine contractions are most powerful. It is impossible to say whether the greater blood supply present at this time causes the increased muscular activity or whether the latter calls for a greater blood supply. It seems more probable that during oestrus both the continuous blush and the characteristic contractions of the uterine muscle are the effects of a modification of general bodily or local conditions.

Source of vascular net of transplants. Since this phenomenon is specific for uterine tissue an attempt to discover the source of the blood vessels in the transplants seemed to be indicated. Had the blood vessels growing in from the iris anastomosed directly with the cut ends of the uterine vessels, or had an entirely new vascular net been formed? If circulation

were reëstablished in the vessels of the transplant, one should expect them to exhibit the characteristic rhythm. If, on the other hand, a new vascular bed were formed in the transplant by vessels that had grown in from the iris it had become modified so that it would blush and blanch, for the iridial vessels do not show a vascular rhythm. Two facts point towards the anastomosis of the ingrowing vessels with the original vessels in the transplant. The first is the extremely rapid vascularization of the transplants. This usually began 24 hours after transplantation and in one case complete vascularization of the transplant and the presence of the vascular rhythm were observed 20 hours after transplantation. This seems too short a time for the formation of an entirely new vascular bed and the acquisition of a rhythm by the newly formed vessels. The second fact is that in a four day transplant most of the vessels, visible microscopically, were found injected with carmine gelatine and no degenerating vessels were seen. Since the degenerating blood vessels in the transplants that fail to vascularize remain visible for more than 4 days it seems that some, if not all, of the original blood vessels were used in the formation of the functional vascular net.

Length of the rhythms. The length of the rhythm varies in different transplants in the same species. The extremes in the lengths of the vascular cycles were 15 and 25 seconds in the guinea pig, 35 and 85 seconds in the rabbit. In the 4 monkeys observed the average lengths of the rhythms were 60, 65, 75, and 80 seconds.

Microscopic structure of the transplants. After 19 months transplants of endometrium into the anterior chamber of the eyes of guinea pigs do not differ in structure from the uterus left *in situ*. Further, the microscopic structure of the transplants undergoes the same changes during the oestrous cycle as the uterus *in situ*. Mitosis are most numerous in the epithelium during the first 4 days of the oestrous cycle and in the stroma from the third to the eighth day. They are found most infrequently in the entire mucous membrane from the eleventh to the fifteenth day. The epithelium degenerates during post-oestrus but is subsequently repaired. During oestrus many leucocytes migrate out of the blood vessels of the transplant into the stroma and thence into and through the epithelium and into the anterior chamber of the eye. During oestrus the entire mucous membrane is very edematous. All these changes occur at the same time and to about the same extent as in the uterus *in situ*.

Effects of lowered blood pressure. If the blood pressure is abruptly lowered to near the lowest point compatible with life, the vascular changes in uterine transplants in rabbits' eyes are not arrested. The edges of the ears were swabbed with xylene and as soon as there was a marked dilatation of the marginal veins they were cut and from 40 to 80 cc. of blood withdrawn from each of 6 rabbits. When the large amounts of blood were

withdrawn the amount of time in partial and complete blanch was lengthened, but the greatest reduction in blood pressure obtained by this method did not greatly affect the rhythmic vascular changes.

DISCUSSION AND SUMMARY

Although cyclic vascular changes have been described in other tissues they have all been quite different in many respects from the ones found in the uterus. The evidence presented seems to indicate that these vascular changes are specific for uterine tissue, at least in a quantitative sense. If similar rhythmic vascular changes do occur in other tissues it is certain that they do so only to a far lesser extent. The facts observed indicate that the vascular rhythm is inherent in uterine tissue.

The presence of the rhythmic vascular changes in the uterus of the monkey, would lead one to suspect that they might also occur in woman. It is difficult to understand how rhythmic vascular changes in the human uterus can have escaped detection were it not for the fact that the only authors who have published descriptions of the living human uterus are Schroeder, Hinricks, and Kessler, (1926), and Schochet (1929).

The Schochet method of transplantation seems to be an especially satisfactory one, for the transplants persist without any apparent change for 23 months. This long persistence may possibly be explained by the fact that no fibrous capsule develops around successful transplants in the anterior chamber of the eye. This may be due to the absence of injury to the actual site of implantation with consequent absence of stimuli for the development of scar tissue and to the rapid removal of catabolic products by the aqueous humor which may remove stimuli to the development of a limiting capsule.

The persistence of the vascular rhythm for 30 to 60 days, in transplants, after the removal of the ovaries seems to indicate that it is not directly under the control of follicular hormone. It seems more likely that it is an inherent characteristic of uterine tissue that only manifests itself when the uterus is in an adult or reproductive state. The persistence of uterine transplants for 23 months without signs of degeneration in non-castrated males and their degeneration accompanied by the disappearance of the vascular phenomenon within 2 months after castration, seems to add evidence in favor of this hypothesis. That castration will cause the degeneration of uterine transplants in male hosts suggests that the female sex hormone described in male urine by Laqueur (1929) may be elaborated by the testicles.

These rhythmic vascular changes seem to be related to the functional activity of the gonads; appearing at sexual maturity, disappearing after spaying or castration and responding to replacement therapy.

They are also, at least temporally, related to the process of ovulation

for the period of continuous blush occurs during the final growth of the follicle.

Since the period of continuous blush can be induced by the injection of follicular hormone and since the length of the period is roughly proportional to the amount of hormone injected, it seems probable that the induction of the period of continuous blush may be used as a test of the amount of follicular hormone present in the blood stream.

Since the period of continuous blush is induced in transplants in noncastrated males by the same amount of follicular hormone as in females, there seems to be no antagonism, at least in this one respect, between follicular and testicular hormone.

The fact that two transplants in the same eye have independent rhythms seems to exclude general bodily changes as a cause.

Since pure endometrial transplants exhibit the vascular rhythm they are not caused by passive compression of the blood vessels by the contractions of the longitudinal or circular muscle of the uterus.

Modifications of the vascular rhythm seem to be governed by a hormonal rather than a nervous control.

Transplants of uterus into the anterior chamber of the eyes of guinea pigs do not become altered in structure from the uterus *in situ*, and their microscopic structure undergoes the same changes during the oestrous cycle as the undisturbed uterus *in situ*.

These rhythmic vascular changes occur in guinea pigs, rabbits and monkeys (Macacus rhesus).

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THE INTERRELATIONSHIP BETWEEN BLOOD SUGAR, BLOOD CALCIUM, AND BLOOD COAGULABILITY[1]

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Several factors are known to affect the coagulation time of the blood. It is a well established fact that there exists a definite relationship between blood sugar content and blood coagulability. This relationship, furthermore, does not depend on the manner in which the hyperglycemia is produced, the resultant shortening of coagulation time taking place whether the blood sugar level is raised through carbohydrate ingestion, glucose injection, or the administration of substances which produce hyperglycemia centrally or peripherally.

Wright (1894) was among the earlier workers to observe that the coagulation time is decreased after meals. Mills (1923) observed a 30 to 40 per cent shortening of the coagulation time about one hour after each meal. Epinephrine, which causes a hyperglycemia, also produces a shortening of the coagulation time, as shown by Cannon and Gray (1914), Grabfield (1916), Tanaka (1928), and others. The parallelism between coagulation time and blood sugar holds also in hypoglycemia, as shown by Svec (1930), who observed a lengthened coagulation time following the injection of insulin. Stuber and Lang (1926) found that substances which retard glycolysis also retard coagulation of the blood. Partos and Svec (1927a, b, 1928) used numerous methods of increasing the blood sugar level in rabbits, and found a parallelism between the blood sugar and coagulation time in all cases. Glucose therapy has been advocated clinically by Schreiber (1913), Kehr (1914), Walters (1921), and Ravdin (1929, 1930) for the purpose of decreasing the coagulation time, especially in lesions of the biliary tract where the coagulation time is prolonged.

The actual mechanism by which the blood sugar affects the coagulation time, however, has not been apparent, since there is no fundamental direct relationship between the cause and the effect. It appeared likely, there-

[1] The experiments reported in this paper form the basis for a thesis presented by Donald W. Cowan in partial fulfillment of the requirements for the degree of Master of Science at the University of Minnesota (May, 1931). A summary of the findings herein reported was read before the Minnesota Branch of the Society for Experimental Biology and Medicine, and published in the Proc. Soc. Exper. Biol. Med., 1930, xxvii, 950.

fore, that some intermediate factor was concerned in the relationship between blood sugar and coagulation time. In this paper we are reporting the results of a series of experiments on both normal and jaundiced dogs, which received intravenous injection of glucose, the blood being tested for sugar, calcium and fibrinogen content, and coagulability at stated intervals before and after the injection.

METHODS. Coagulation time was determined 1, by the method of Lee and White (1913), and 2, by the following modification of Wright's method (1893)[2]: Glass tubes, 40 mm. long and 2 mm. internal diameter are placed horizontally so that one end of each projects over the edge of a table. Each tube is half filled with blood drawn by venepuncture into a glass syringe previously rinsed with physiological sodium chloride solution. Coagulation is tested at half-minute intervals by tilting one of the tubes into the vertical position. The end point is taken when the blood fails to run down the sides of the tube. Each tube is tested once only. The results obtained by this modification were checked against those obtained by the Lee and White method in a large number of determinations. The two methods usually checked within ½ minute, often the results were identical. Rarely one minute's difference was observed, especially with jaundiced animals, where the sedimentation was very rapid. In such cases the endpoint is much more readily recognized by the modified Wright's method, and in later experiments this method was used exclusively.

Serum calcium determinations were made by the method of Kramer and Tisdall (1921) and Tisdall (1923). Fibrinogen was determined by the technique of Wu (1922), except that the standard used was a solution of fibrinogen rather than one of tyrosine, this modification having been suggested by Gibson (1931). The method of Folin and Wu (1920) was used for the determination of blood sugar. In the earlier experiments on jaundiced animals the actual bilirubin content of the plasma was determined by Thannhauser and Anderson's (1921) modification of Van den Bergh's method. In the later experiments the "icterus index" was determined.

EXPERIMENTAL. The animals were usually fasted over night. The animals were trained to lie quietly, lightly tied to an animal board, great care being taken to avoid any exciting influences which might affect the animal's metabolism. Venepuncture was made on the jugular vein, using a sharp needle of large bore and 20 cc. syringe previously rinsed in 1 per cent sodium chloride solution. In any case where the needle did not enter the vein at the first attempt, the needle was withdrawn, and needle and syringe thoroughly rinsed before repeating the attempt. The stopwatch was

[2] The authors desire to express their indebtedness to Dr. A. D. Hirschfelder for the method here described, which he has employed with considerable success in laboratory demonstrations for a number of years.

tripped at the first appearance of blood in the syringe. After removal of a control sample of blood, the measured amount of glucose was injected into the saphenous vein at the rate of 5 cc. per minute. Blood samples were removed 5, 30, 60, 90, 120, 150, 180 and 240 minutes after completion of the glucose injection. Jaundice was produced in the dogs by ligation and section of the common bile duct, plus cholecystectomy. The jaundiced animals lived for periods up to 4 months after operation. Some of the

TABLE 1
Normal dogs

WEIGHT	DOSE OF GLUCOSE	COAGULATION TIME			BLOOD CALCIUM		
		Initial	Lowest	Per cent decrease	Initial	Highest	Per cent increase
kgm.							
15.8	30 cc. of 50%	6½	3	53.8	10.7	11.7	9.4
16.0	50 cc. of 50%	10	8	20.0	11.0	12.1	10.0
15.3	50 cc. of 50%	12½	8	36.0	9.6	11.2	16.7
19.0	75 cc. of 50%	11	3	72.7	10.0	14.2	42.0
22.0	80 cc. of 50%	6	4	33.3	10.5	11.3	7.8
17.0	60 cc. of 50%	12	4½	62.5	8.0	10.4	30.0
19.1	60 cc. of 50%	10	4	60.0	9.3	10.3	10.8
20.3	80 cc. of 50%	15	3	80.0	9.3	11.0	18.3

TABLE 2
Jaundiced dogs

WEIGHT	DOSE OF GLUCOSE	COAGULATION TIME			BLOOD CALCIUM		
		Initial	Lowest	Per cent decrease	Initial	Highest	Per cent increase
kgm.							
14.4	60 cc. of 50%	13.0	4½	65.3	8.8	10.8	22.7
17.1	60 cc. of 50%	11½	9	21.7	9.1	9.8	7.9
13.4	30 cc. of 50%	18	7	61.1	9.7	11.9	22.7
12.0	60 cc. of 50%	12½	8	36.0	9.0	11.2	24.4
22.0	80 cc. of 50%	11½	5½	52.1	10.5	12.3	17.5
22.1	80 cc. of 50%	10½	4	61.9	11.0	16.4	49.0
22.1	80 cc. of 50%	11	4	63.6	11.2	14.8	32.1

animals were forcibly fed with milk and syrup to offset the anorexia and attendant marked loss in weight; others were allowed to eat only what they wished. Four dogs developed ascites and increased collateral circulation on their anterior abdominal walls during the course of the obstruction. Only one jaundiced dog had a coagulation time which was increased to any degree over the normal figure. The calcium and fibrinogen values for the jaundiced animals were within normal limits.

Table 1 summarizes the results of 8 experiments on normal dogs, and table 2 gives the results obtained in 7 similar experiments on jaundiced dogs. In all, 28 experiments were carried out.

We obtained a lowering of coagulation time after sugar injection in 76 per cent of the experiments on normal animals, and in 100 per cent of the experiments on jaundiced animals; moreover, the coagulation time was decreased 50 per cent or more in 47 per cent of the normal dogs and in 73 per cent of the jaundiced ones. These results are really quite striking. How does this increased coagulability come about? The nearly absolute parallelism between blood sugar and blood coagulability that Partos and Svec believe to exist, has not proven out in this laboratory; for the increased coagulability after sugar injection does not occur simultaneously with the increased blood sugar, but lags behind for from 15 minutes to 1½ hours after the injection. Nor does the coagulability decrease with the blood sugar, but in many cases stays up several hours after the blood

TABLE 3

Dog 12, jaundiced, weight 12 kgm., poor nutrition, not forcibly fed

TIME	COAGULATION TIME	BLOOD SUGAR	SERUM CALCIUM
	minutes	*mgm. per cent*	*mgm. per cent*
Fasting	12½	70	9.0
Injection of 50 cc. of 50% glucose intravenously in 8 minutes			
5 min.	13	136	9.7
30 min.	6	96	11.2
1 hour	8	66	10.9
1½ hour	11	74	9.6
2 hours	12	67	9.2

sugar has again returned to normal. These facts would seem to point to something other than the actual blood sugar value as the controlling factor. Ravdin reported no increase in blood fibrinogen after sugar injection. A few experiments done in this laboratory support this finding.

In a search for other changes in the blood after sugar infusions, which might explain the change in coagulability, serum calcium determinations were made. It was found that after intravenous injection of glucose the blood calcium increased with striking constancy. Furthermore, when the calcium, sugar, and coagulability values of the blood were charted together, it was seen that the calcium curve was much more nearly parallel to the coagulability curve than was the curve of the blood sugar. These results, then, throw a new light on the subject of blood sugar and blood coagulability. Table 3 shows the typical results obtained.

Discussion. It seems clear, from the above experiments, that the important effect of raising the blood sugar in the attempt to decrease the

coagulation time is not the rise in the sugar level itself, but the increase in blood calcium which follows the hyperglycemia; for as already stated, the calcium goes up with a curve parallel to that of blood coagulability and remains up while the coagulation time remains shortened. On the other hand, the sugar is already on its way back to normal before the coagulability is increased to any degree, and it has reached normal or below long before the coagulation time begins to lengthen again.

The mechanism by which this increased blood sugar produces the hypercalcemia is not yet understood. The blood sugar and calcium levels, however, must be very closely interrelated for Minot and Cutler (1928) have shown that the administration of calcium salts raises the blood sugar level, and we have confirmed this finding in this laboratory. The effectiveness of the increased calcium called forth by the glucose injection in reducing the coagulation time would indicate that this freshly released calcium was in a more readily available form for taking part in clot formation than that already present in the blood particularly in cases of jaundice.

We found repeatedly in our experiments that the characteristic heightened and prolonged sugar curve after sugar injection which is seen in a subject with liver damage does not hold if that subject has been without sufficient carbohydrate diet for any length of time. In this situation, intravenous glucose has little or no effect, and if it does have an effect, it is only for a very brief period of time. Under such conditions the liver appears to take up the glucose with astonishing rapidity. However, that same subject, if forcibly fed for a week with high carbohydrate diet, will respond nicely to sugar infusion, the sugar curve will be of the characteristically prolonged type, and the coagulability will likewise increase and remain high for a number of hours after injection.

CONCLUSIONS

1. Coagulation time of the blood in living animals may be decreased by injections of glucose.

2. Intravenous glucose is more effective in decreasing the coagulation time in jaundiced dogs than in normal dogs, not only in percentage of successes, but also in the degree of shortening.

3. Raising the blood sugar through glucose infusions results in a heightened blood calcium, as well as a heightened blood coagulability.

4. The blood calcium more closely parallels the blood coagulability curve than does the blood sugar curve, after intravenous glucose injection.

5. High carbohydrate forced feeding aids the coagulation-time decreasing effect of intravenous glucose in jaundiced animals with liver damage.

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EFFECT OF TOTAL ABSENCE OF FUNCTION ON THE OPTIC SYSTEM OF RABBITS

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The determination of the rôle that function plays in the morphogenetic development of the central nervous system is a fundamental problem that has engaged alike the attention of physiologist, neurologist, and experimental zoölogist. The optic system, due to its more or less discrete nature, has been a favorite one for experimentation on this subject. The work on mammals has been confined for the most part to the extirpation of eyes and the sewing of eyelids in an attempt to remove function from the visual pathways. Eye excision experiments have been performed by many investigators. This technic, however, is complicated by secondary degeneration, and only neurones of the third order (superior colliculus, lateral geniculate, and pulvinar) remain for study. The neurones of the first and second order (bipolar and ganglion cells, respectively, of the retina) are removed by the section of the optic nerve and cannot be examined for the effect of lack of stimulation. Sewing eyelids in rabbits (von Gudden) and in cats and dogs (Berger) does not exclude function completely since the eyelids of these animals are translucent to bright light. The very nature of these experiments, then, precludes an accurate interpretation of results. To estimate the part occupied by peripheral sensory field on the post-natal growth and differentiation of the visual apparatus in mammals, a method was sought which would leave the system under observation anatomically intact and at the same time allow the factor of complete lack of function to be the only variable.

EXPERIMENTAL PROCEDURE. A dark room was improvised which could be entered only through a series of three doors. Sensitive panchromatic photographic films exposed repeatedly for forty-eight hour intervals failed to record any light. This method of light determination is more sensitive than the photoelectric cell. The room was well ventilated and supplied with a large cage and a small light-proof box into which the rabbits could be put for the short time needed daily for feeding and refuse disposal. A healthy pregnant rabbit near term was placed in the room and allowed to give birth to her litter (of nine). A liberal diet containing cod liver oil was given to the mother, and to the young after weaning.

Each day the mother was removed to the sunlight for two hours, and after six weeks was taken from her young. For the duration of the experiment, six months, no one entered the room except with the writer.

When the young were nine days old and before their palpebral fissures were patent, three of the dark room animals were removed to a dimly lighted room for operation. While the right eyes were constantly well protected by a light-proof blinder attached firmly over them, the left eyes in two animals under ether anesthesia were enucleated, and in the remaining rabbit the left eyelids were sewed after the free lid margins were cut away. The procedure took less than five minutes in each instance and the animals were returned promptly to the dark room where recovery was uneventful.

The rabbits were sacrificed by asphyxia in the dark room at varying intervals. One was transferred to the light at the end of five months for a study of its optic reflexes and behavior. Three, including one of the enucleated, were allowed to remain the full six months. All animals were weighed, measured, and examined closely for evidence of deficiency disease, and the viscera and long bones preserved for study. The brains and eyes were immediately removed and fixed either in a mixture of formalin, alcohol, and glacial acetic acid, or in osmic acid and potassium dichromate for the Golgi triple silver impregnation. The brains and eyes were measured and photographed. A motion picture was taken of the behavior of the rabbit removed to the light.

Controls. A litter of rabbits born from the mating of the same buck and doe as used in the dark room experiment was raised in the light and its members killed at the same age intervals as the experimental rabbits. In addition, a number of control eye extirpation and eyelid sewing experiments were performed on very young animals raised in the light. In each case, the left side was used, and the right remained as a control since there is almost complete decussation in the rabbit's chiasm (Barker). The tissues were handled in a manner identical to that used on the dark room material.

The results of excision of an end-organ, the partial elimination of function, and the complete absence of function could thus be determined.

GROSS ANATOMICAL RESULTS. In a preliminary report, the results of the first three months of the dark room experiments were given. The six month rabbits also showed excellent growth in size and weight when compared with their controls, and no evidence of deficiency disease was found. The eyes of the unoperated dark room animals presented no differences from those of the controls in size or transparency of the cornea or crystalline lens, the extra-ocular muscles, or, indeed, in any part of the accessory optic apparatus. There were no differences in the optic nerves. Careful observations and measurements of the superior colliculi, lateral genicu-

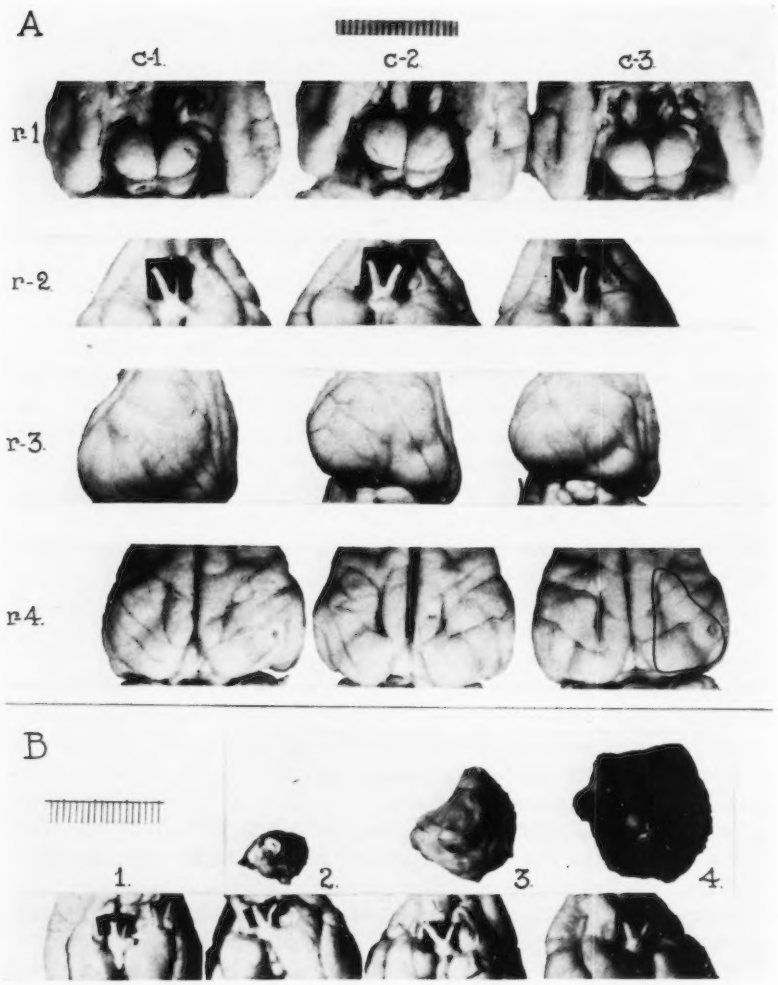

Fig. 1. Photographs of sixth month brains. *c*, column; *r*, row. A. *c-1*, dark room unilateral eye excision; *c-2*, dark room unoperated; *c-3*, light control; *r-1*, superior colliculi; *r-2*, optic nerves; *r-3*, lateral view occipital cortex; *r-4*, dorsal view. Penned area in *c-3*, *r-4* is the striate region in the rabbit. Note reduced size of the colliculus in the operated dark room animal, and the lack of cortical changes in any of the brains. Scale in millimeters.

B. *1*, Unilateral eye excision, control light experiment. *2*, *3*, and *4*, unilateral eyelid sewing light control experiments. Note relation between size of eyes and corresponding optic nerves. Scale in millimeters.

lates, and occipital cortices likewise gave no disparities not equally demonstrated in the group of normal controls (fig. 1-A, *c-2* and *c-3*).

The dark room animals with left eyes excised soon after birth (fig. 1-A, *c-1*) showed the typical degeneration changes in the cerebral stump of the left optic nerves, right optic tracts, and the right primary optic centers,—so ably described by von Gudden. No gross cortical changes were evident. The unoperated tracts could not be differentiated from the controls reared in the light (fig. 1-A, *c-3*). In these dark room enucleation experiments, then, the two procedures of removal of an end-organ and removal of function contrast sharply in the same brain. The invalidity of the former technic as a physiological method of inquiry into effects of non-use of a peripheral sensory organ on the central pathway and centers is apparent.

The dark room rabbit with eyelid sewn soon after birth was killed at the end of two months. The lid was completely ankylosed. No differences were demonstrable in comparing the eyes and brain of this animal with a two month unoperated control raised in the light. Nor were there any dissimilarities between the two sides of this experimental animal's brain.

The control eye extirpation experiments (fig. 1-B, *1*) gave the same typical von Gudden degeneration picture of the dark room enucleated rabbits. The control eyelid sewing experiments, though, differed in the following way. A series of eyes were obtained varying from one normal in size and structure (fig. 1-B, *4*) to one so scarred and atrophic that it could hardly be recognized as an eye (fig. 1-B, *2*). This is not hard to understand inasmuch as the operations were not always successful in avoiding damage to the lachrymal apparatus, and in some instances where the cornea was scarred in addition to the lid sewing, the cautery accidentally perforated into the anterior chamber. The interesting part of these experiments is that the optic nerves and superior colliculi are roughly proportional to the size of the eyes. No cortical changes were found.

Microscopic results. *Eyes and retina.* Microscopic examination of the eyes of the sixth month dark room animals showed no alteration in the structure of the cornea, lens, iris, or ciliary apparatus when these were compared with the controls.

Meridional sections of the retinae passing through the optic nerves were examined with hematoxylin-eosin, iron-hematoxylin, toluidin-blue, and triple silver impregnation stains. The total thickness of the retina and the thicknesses of the fiber and cell laminae differ considerably in the several parts of the retina, varying with the distance between the optic papilla and the ora serrata, the medullated fiber region, and the visual streak. These features of the normal rabbit's eye have been accurately described by Davis and others. Measurements of corresponding retinal

areas midway between the papilla and ora serrata and not through the visual streak or medullated fibers made with a calibrated eyepiece filar micrometer gave the following averages for several hundred determinations:

LAYER	6 MO. DARK (EXPTL.)	6 MO. LIGHT (CONTROL)
Optic fiber, ganglion cell, and internal molecular layers	37 micra	39 micra
Bipolar (int. nuclear)	17 micra	18 micra
Outer molecular	8 micra	7 micra
Outer nuclear	26 micra	27 micra
Rods and cones	24 micra	24 micra
Total thickness of retina	112 micra	115 micra

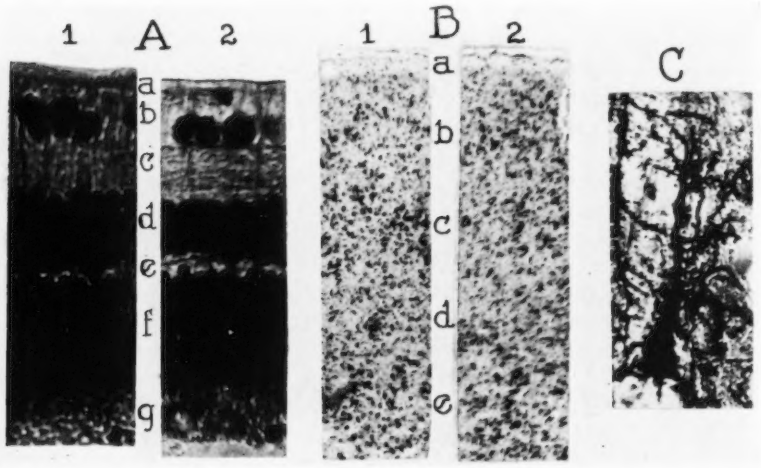

Fig. 2. Photomicrographs. A. Retinae of full term experimental, *1*, and control, *2*, rabbits. *a*, axone layer; *b*, ganglion cell layer; *c*, inner molecular layer; *d*, inner nuclear; *e*, outer molecular; *f*, outer nuclear; *g*, rods and cones, *H-E* stain.

B. Superior colliculi of sixth month experimental, *1*, and control, *2*, brains. *a*, stratum zonale; *b*, stratum griseum superficiale, outer layer of small cells; *c*, stratum griseum superficiale, inner layer of large polygonal cells; *d*, stratum medullare superficiale; *e*, stratum griseum intermedium. Note thickness and regularity of lamination. Toluidin-blue stain.

C. Small pyramidal cell of experimental striate cortex. Golgi stain.

These figures agree closely with those of Davis. Measurements through the medullated fibers and visual streak gave no appreciable differences between the control and experimental material. Also no changes were seen in the regularity of retinal layers in the experimental eyes. Photo-

micrographs of the retinal layers to show their thickness and regularity are given in figure 2-A (*1*, experimental; *2*, control).

Ganglion cell counts and measurements were made on over a hundred sections in corresponding retinal areas near the optic papilla on the temporal side, and through the visual streak. Only the larger cells with nuclei visible were measured. The results are as follows:

	AVERAGE NO. OF CELLS PER FIELD (LEITZ 5 OBJECTIVE, 10 EYEPIECE)	AVERAGE SIZE OF LARGER GANGLION CELLS
6 mo. dark (exptl.)	43 cells	15.3 micra
6 mo. light (control)	41 cells	14.7 micra

Thousands of cells of the ganglion layer were studied under oil immersion in toluidin-blue preparations for the amount and character of their Nissl material, and no alterations were found in these respects in the experi-

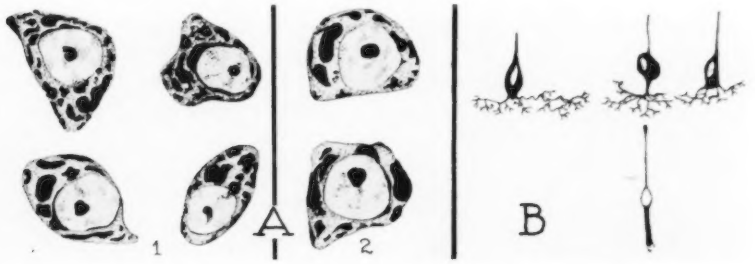

Fig. 3. Artist's drawing. Retina. A. Experimental retina ganglion cells, *1*, and controls, *2*. Note Nissl granules. Toluidin-blue stain.

B. Experimental retinal bipolar and visual cells. Note abundance of dendrites. Silver stain. See text.

mental retinae when compared with the controls. Figure 3-A shows the artist's drawing of typical retinal ganglion cells and their extra-nuclear chromatin. Many scores of Golgi triple silver impregnation preparations of the retinae were examined for the differentiation of cell processes. These as well as the iron-hematoxylin sections failed to show any difference in the degree of cell development of the experimental retinae when compared with the controls. The artist's accurate drawing of a group of bipolar cells and a visual cell (fig. 3-B) shows no lack of dendrites in the dark room retina. The axone terminals of the bipolar cells are not shown because the ganglion cell and internal molecular layers took the silver precipitate too heavily for study except at the optic papilla where the abundance of axones offered protection. In like manner, no changes were seen in the pigment layer or in the structure of the optic papilla.

The small differences noted in the tables given above are well within the percentage of error of measurement, and the differences found within each group were larger than those found between groups.

Optic nerves. Cross sections of the experimental and control optic nerves presented no dissimilarities in cross section area. Osmic acid preparations showed no changes in myelinization.

Superior colliculi. When the sixth month dark room animals were compared with their controls in respect to the regularity and thickness of cell and fiber lamination, no significant differences were found (fig. 2-B). The strata widths were measured with the micrometer in corresponding regions, taking into consideration that the relative thicknesses of the layers differ in the several parts of the colliculus, and that the architectonics are much altered by the angulation of the plane of section. Particular attention was paid to the stratum griseum superficiale (terminology of Winkler and Potter) since this is probably the most important cell layer of the optic system in the colliculus for study, receiving as it does the endings of the neurones of the second order through the optic nerve, and being mainly if not solely visual in its afferent connections. Cell counts were made on corresponding areas and reported here as number of cells per field of a micro-projector apparatus. The averages from scores of determinations are as follows:

	AVERAGE THICKNESS STRATUM GRISEUM SUPERFICIALE	AVERAGE NUMBER OF CELLS PER MICROPROJECTOR FIELD
6 mo. dark (exptl.)	870 micra	292 cells
6 mo. light (control)	850 micra	283 cells

The stratum medullare superficiale (fig. 2-B, *d*) which contains the incoming optic fibers is not as well defined for micrometer measurement. Careful study of iron-hematoxylin preparations revealed no differences, however, in this layer either in regard to the amount of fibers or their mode of distribution. Nor did the deeper cell and fiber laminae disclose any alterations when compared with the controls.

Toluidin-blue preparations for the study of Nissl granules showed the same amount and character of substance in the experimental and control colliculus cells. Figure 4-A shows the artist's drawing of the larger cells with abundant extra-nuclear chromatin. There were no dissimilarities in cell size or nucleus to cytoplasm ratio. Many excellent Golgi preparations were examined for cell processes and as shown in figure 4-B there was no lack in the experimental material of normal dendritic development.

The colliculus of the sixth month dark room enucleation experiment (fig. 5-C) confirmed on microscopic examination what the gross results

suggested. Whereas the unoperated side could not be told from the controls raised in the light, the operated side gave the changes reported in the literature by previous workers. It need only be mentioned here that as noted by Cajal the stratum zonale is unaffected; the principal changes are not in the cells (if indeed these are altered at all) but in the substantia gelatinosa of the endings of the optic nerve. These results again repudiate the interpretation of effects of sense organ removal as being due to absence of peripheral stimuli.

Lateral geniculates. In the rabbit the structure of these bodies is fairly well known except for some of the fiber connections of the ventral nucleus.

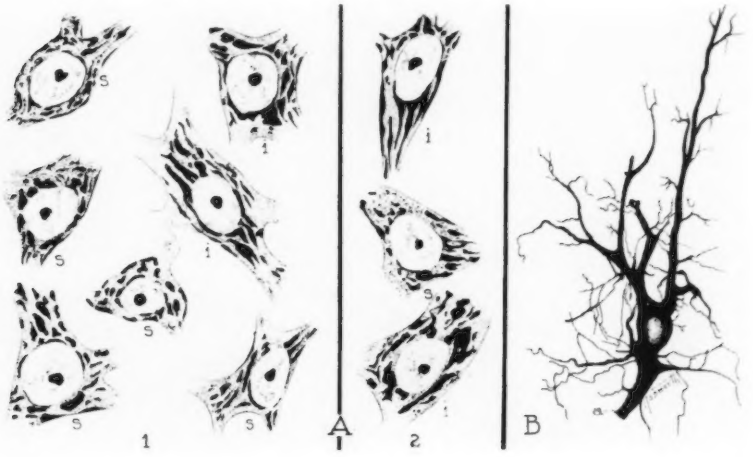

Fig. 4. Artist's drawing. Superior colliculi. A. Experimental, *1*, and control, *2*, cells. *s*, cells from superficial cell layer; *i*, cells from intermediate cell layer Note Nissl granules. Toluidin-blue stain.

B. Cell from superficial cell layer experimental colliculus. *a*, axone. Note abundance of dendrites. Golgi preparation.

They are said to receive around 80 per cent of the axones of the ganglion cells of the retina through the optic nerve and tract. The dorsal nucleus, which is thought to be the more recent phylogenetically, relays the representation of the retina to the area striata of the occipital cortex, and contains cells whose axones form the optic radiation of the internal capsule. That these cells represent neurones of the third order in the optic conduction pathway has been emphasized by Barker and others. These dorsal nuclei cells were thus considered especially important ones for this study. No discernible differences were found in the size or shape of the dorsal or ventral nuclei, nor were there any changes in the "laminations" of the

dorsal nucleus (fig. 5-A, *2*). The character and amount of Nissl substance were determined by oil immersion study of hundreds of cells of these nuclei in toluidin-blue preparations, and as shown by the artist's accurate drawings (fig. 6-A) no disparities were seen in comparing the experimental with the control sections. No differences were found in the relative num-

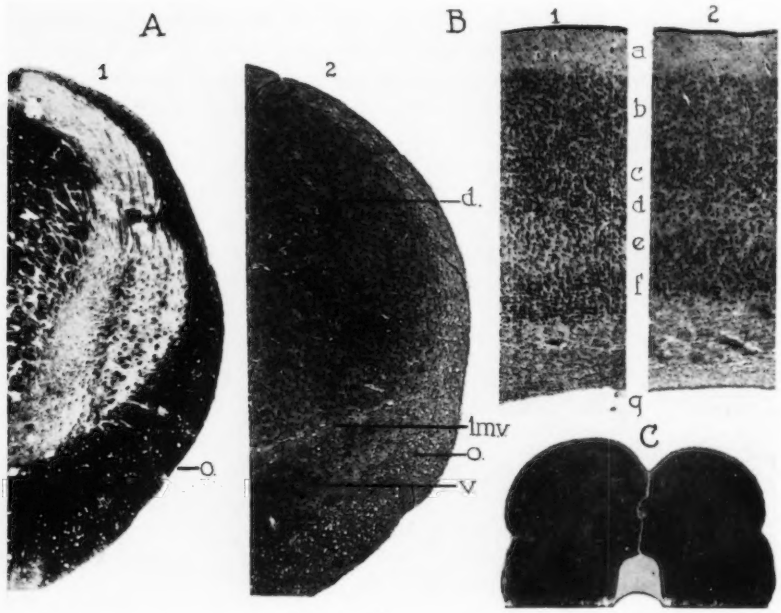

Fig. 5. Photomicrographs. A. Experimental lateral geniculate body sixth month brain. *1*, iron-hematoxylin; *2*, toluidin-blue; *d*, dorsal nucleus; *v*, ventral nucleus; *o*, optic tract on lateral aspect thalamus; *l.m.v.*, lamina medullare ventralis.

B. Experimental, 1, and control, 2, striate cortices. *a*, lamina molecularis; *b*, lamina granularis externa; *c*, lamina pyramidalis; *d*, lamina granularis interna; *e*, lamina ganglionaris; *f*, lamina multiformis; *g*, caudal tip lateral ventricle. Note thickness and regularity of lamination. Toluidin-blue stain.

C. Superior colliculi of unilateral eye excision dark room brain. Smaller colliculus on side opposite to operation. Gross specimen seen in figure 1-A, *c-1*, *r-1*, Toluidin-blue.

ber of cells or their ratio of nucleus to cytoplasm. Many Golgi preparations of this region were observed for cell differentiation and type, and here again no lack of normal dendritic development or cell types characteristic of the geniculate nuclei was discovered in the experimental material. A drawing of one of the dorsal nuclei cells stained with the silver method is shown in figure 6-B.

The dark room eye enucleation experiments, however, show a difference between the two sides of the same brain. The side whose anatomical connections were untouched could not be told from its control reared in the light. The side whose optic relations were severed by eye excision presented changes reported by other investigators. It is sufficient to state that the changes observed are mainly in the incoming optic tract fibers, the cells not being altered. This, again, as in the colliculus, shows the difference between removal of an end organ and absence of function.

Pulvinar. This diencephalic optic center likewise demonstrated no alterations when studied in the manner outlined above, although no silver preparations of this nucleus were made.

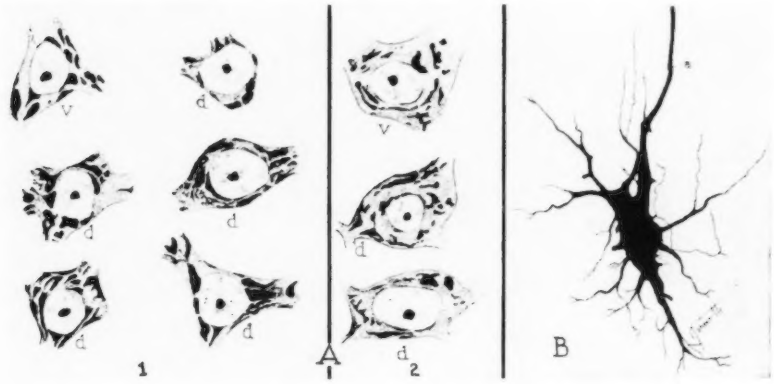

Fig. 6. Artist's drawing. Lateral geniculates. A. Experimental, *1* and control, *2*, cells; *d*, dorsal nucleus cells; *v*, ventral nucleus cells. Note Nissl granules. Toluidin-blue.

B. Experimental dorsal nucleus cell. *a*, axone. Golgi preparation.

Occipital cortex and area striata. The location and histological features of the striate area in the rabbit's cortex are well understood (Winkler and Potter; Putnam and Putnam). An examination of the thickness of the visual cortex in the experimental and control brains gave the following averages for fifty estimations made in the striate area overlying the caudal tip of the lateral ventricle:

	6 MO. DARK (EXPTL.)	6 MO. LIGHT (CONTROL)
Lamina molecularis	276 micra	282 micra
Lamina granularis externa to and including lamina multiformis	1,617 micra	1,599 micra

Similar measurements made on other representative parts of the occipital cortex showed no appreciable differences between the control and dark

room brains. A study of the regularity of lamination in the striate cortex likewise disclosed no alterations in the experimental material (fig. 5-B). Micrometer measurement of the thicknesses of each particular layer was found to be a very difficult and uncertain task, and the error too great to give dependable results. Careful examination of toluidin-blue and iron-hematoxylin preparations, however, revealed no disparities in the thicknesses of the various laminae.

Cell counts made in analogous regions demonstrated no significant differences in the number of cells in the various parts of the experimental

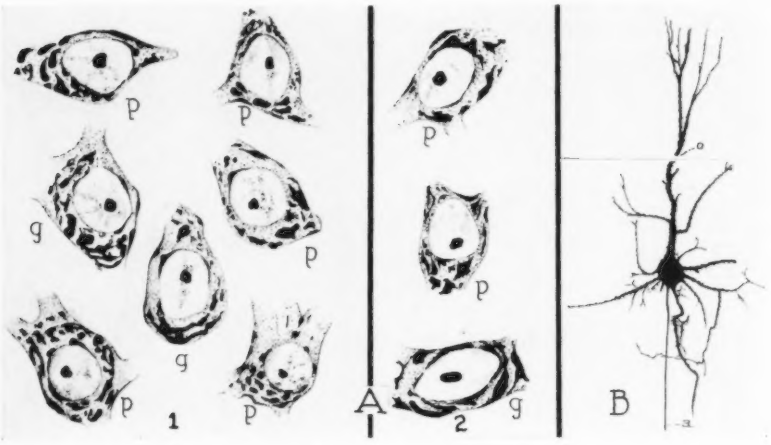

Fig. 7. Artist's drawing. Striate cortex. A. Experimental, *1*, and control, *2*, cells. *p*, pyramidal layer; *g*, ganglion layer cells. Note Nissl granules. Toluidin-blue stain.

B. Moderate-size pyramidal cell experimental striate cortex; *a*, axone; *o*, omitted portion of dendrite. Golgi preparation.

striate cortex when this was compared with the controls. One of the typical averages (ten counts) representative of numerous similar counts is as follows:

	6 MO. DARK (EXPTL.)	6 MO. LIGHT (CONTROL)
No. of pyramidal cells per field of microprojector apparatus	325 cells	306 cells

No importance is attached to this difference.

Examination of the cells of the visual cortex in the experimental and control brains by means of Nissl stains showed that most of the pyramidal

and ganglion cells have large nuclei in relation to cytoplasm, and no, or fine and scant, Nissl granules. In these features of extra-nuclear chromatin and nuclear size no dissimilarities were found between the two groups. Some of the larger pyramidal and ganglion cells contain abundant and fairly large Nissl bodies and also the number of these cells do not differ in experimental and control cortices (fig. 7-A, *1* and *2*). The Golgi preparations showed normally developed dendritic processes (figs. 7-B; 2-C) and no dearth of the various kinds of cells seen in silver stains of the visual cortex. No dissimilarities were found when the experimental and control sections were compared in these respects.

A study similar to that outlined above of the cortex of the full term enucleated dark room rabbit showed no differences between the two sides. This is confirmatory of von Gudden and von Monakow.

PHYSIOLOGICAL RESULTS. A rabbit was taken into the light at the end of five months for a study of its behavior and optic reflexes. Directly before removal from the dark room its reflexes to light were tested. The first flash of light caused an immediate eyelid closure, the lid being held closed and the plica semilunaris drawn over the cornea. When the lid was forcibly opened, the pupil contracted after a latent period of five seconds.

The animal was then transferred to an open area illuminated by sunlight and strewn about with various obstacles. While in the bright light it kept its eyes almost completely shut. When in the shade, it kept its lids open. This was rather an unexpected observation that the retina could adapt itself so quickly to the intense stimulation, and allow the animal to display so little photophobia. That the rabbit was an albino lends added significance to this.

The posture of the animal was exceedingly interesting. It walked on a broad base with forelegs, especially, widely spread. Its neck protruded full length in advance of its trunk, and the head was held so low that the snout almost touched the ground.

The rabbit did not avoid obstacles placed in its path. It seemed to depend on cutaneous, olfactory, and auditory cues. It sniffed continuously, and when walking along the side of a wall would turn its head so that the vibrissae of its snout remained in contact with the wall. When placed in a low box, it manoeuvered about, feeling the edge of the box with its front feet, cautiously putting its head and neck over the edge, but not electing to jump the short distance to the ground.

When in the sunlight near a patch of shade, it did not move toward the shade; when in the shade, it avoided moving out into the sun.

For the first hour it kept its ears flat against its back. That this may have been due to fright as a result of the world of new experiences suddenly thrust upon it is suggested by the fact that its respiratory rate was fully twice as fast as that of the controls with whom its behavior was compared.

An hour later, its ears were kept erect, and its respiratory rate had diminished a third.

Its keen sense of smell was demonstrated by holding a piece of carrot several inches from its nose. At this distance the controls ignored the object, but the experimental rabbit protruded its head and walked toward it. Only when the carrot was held much closer did the controls appear to notice it.

During each of the days that followed the animal's transfer to the light, its blink, corneal, and pupillary reflexes were tested and its gait and ability to avoid obstacles were observed. In none of these respects except that of posture could the experimental rabbit be told from the controls. Within one week, its posture improved but even at the end of two months was still detectable as different. Within the first ten days, the animal advanced considerably in its ability to avoid objects in spite of the fact that it was kept all day in a very small cage. By the end of this time, even when frightened into running, it could wind in and out of numerous obstacles without hitting them. Sudden movements of objects near its eyes when due precautions were taken to prevent auditory or cutaneous stimuli from operating appeared to frighten the rabbit, and it would open its eyes widely and turn its head to the direction opposite the object (so as to bring the latter into the field of vision?).

At the end of one month the rabbit would follow with its eyes hand movements made two feet away, would turn to avoid sudden movements of objects toward him, and would jerk its head and body if the open hand was suddenly closed. Proper care was taken in all these tests to avoid other stimuli than visual.

At the end of two months the rabbit was killed and its tissues fixed, stained, and studied as outlined above. No differences, gross or microscopic, were found in any part of the optic system when this was compared with that of the control and full term dark room animals.

Discussion. The magnitude of the problem of the rôle of function in the differentiation of the nervous system makes it necessary to limit this discussion almost entirely to the more important studies in respect to the optic system. No attempt at a systematic or complete account is pretended, and many relevant and interesting data are purposely omitted.

Von Gudden after sectioning the optic nerve or destroying the retina in young rabbits observed an extremely thin optic nerve, grey instead of white, on the operated side; the opposite anterior colliculus much reduced in size; the lateral geniculate little if any changed; the cerebrum unchanged. In other experiments in which he sewed the eyelids of newborn rabbits, the gross anatomical changes were very slight. In commenting on this difference between sewing and enucleation, he stated that light probably reached the eyes through the lids, inferring that if all stimuli were removed a different picture would occur.

Von Monakow after studying the effect of eye extirpation on the primary optic centers noted that secondary degeneration never extended beyond them, and that changes in them were due to lack of molecular substance and end processes of the second nerve incident to the degeneration of the latter, and that the cells did not suffer. Cells that disappeared in the colliculus belonged to the centrifugal system. After many years, however, the main cells of the lateral geniculate showed some reduction in size, but no structural change. If the neurones of the third order are affected so meagerly by absence of the optic nerve it is hardly to be expected that they would be seriously altered by removal of impulses coming over the optic nerve.

Berger, using lid sewing on young cats and dogs, and examining them several months later, found no gross or microscopic changes in any part of the visual system except the occipital cortex. Here he noted narrower convolutions, imperfect sulci, and a thinner cortex. Microscopically there was no difference in cell numbers in the optic cortex, but a crowded arrangement of the small pyramidal cells, large nuclei in relation to cytoplasm, and fewer and finer Nissl granules. These features were interpreted as arrested development due to lack of function. He admitted that all stimulation was not removed by his procedure and accounted for the changes being found only in the cortex by assuming that the indefinite and weak stimuli are sufficient to keep the retina and primary centers normal, but that more definite and intense stimuli are needed by the cortex for development. He suggests that complete lack of light would result in marked atrophy of the optic nerve and primary centers.

The effect of eye removal, optic atrophy, and congenital micro- and anophthalmos on the cortex is a moot question. The experimental and clinicopathologic studies have not given consistent findings, and the results seem to vary with the investigator, the animal used, its age at the time of operative interference, and the length of time the experiment is allowed to continue. In bilateral enucleation in newborn rabbits neither von Gudden nor von Monakow could find any atrophy of the cortex; but the latter worker found an equal reduction of the gray and white matter diffusely throughout the occipital cortex of dogs six months after bilateral extirpation at birth. Tanzi, on the other hand, found changes in bilaterally excised young dogs relatively soon after operation, noting a decrease in size of nerve cells, and a reduction in the amount of nerve fibers and molecular substance, these changes being localized in the medial occipital convolution. The above mentioned works were done during the time that the exact localization of the visual area in the cortex was still under dispute.

Steinetz removed the eyes of larval frogs and noted the marked reduction in the size of the optic lobes after metamorphosis, showing in his figures that there was also a marked reduction in the number of cells in the opticus layers. Larsell recently repeated these experiments, enucleating eyes

unilaterally in 18–22 mm. and 30 mm. larvae of tree frogs. From more detailed cell studies he concludes that the absence of normal optic stimuli from the periphery or factors directly attributable to these stimuli accounts for the reduction in the number and size of cells, and their lack of normal dendritic development.

Donaldson described the brain of the blind-deaf mute, Laura Bridgman, and attributed the differences between the two sides of the occipital cortex to the fact that the left eye was completely blind at two years of age, but the right eye perceived light until eight. He says, "The conservation value for the nerve centers of even such weak stimuli has long been recognized and it is but natural therefore that the occipital lobe chiefly concerned with the right eye should be better preserved than the other whose development was presumptively arrested earlier during the years most important for growth." "The persistence of vision, though in a defective form, is still of great importance to the full development of the visual cortex . . ." ". . . the disturbance in the cortex is probably to be looked upon much more as due to an arrest of growth following the removal of the normal stimuli than to a continuation of the degeneration into the hemispheres." In remarking on the changes in the auditory cortex of this brain, the same writer says, "The disturbance here is most probably due to early and long continued lack of normal excitation, for the cortical cells in the sensory area are peculiarly dependent for their proper development on the special sense with which they are associated."

Workers in experimental zoölogy have studied the processes and factors involved in the proliferation and differentiation of the cells of the central nervous system. Most of this work has been performed on larval amphibia. The important contributions of Burr, Detwiler, Coghill, and many others are too numerous to enumerate here. Herrick in his review of the morphogenetic factors in the differentiation of the nervous system mentions many of these experiments and calls attention to three stages in development,—cell proliferation before maturity or function, cell differentiation or growth to functional efficiency, and a third stage in which further development may be influenced by actual transmission of nervous impulses. Burr, in an excellent short summary of the mass of difficult experimental data states that on the whole the weight of the evidence so far presented in the literature argues against the concept that functional activity is a potent factor in the morphogenesis of the central nervous system.

In regard to the experiments presented in the body of this paper, the eyelid sewing and enucleation experiments confirm the results obtained by von Gudden and von Monakow. In lid sewing, providing that the lid margins are not cut away too liberally and that no damage is done to the lachrymal apparatus so that infection, retention of secretions and pressure

atrophy do not intervene, no anatomical changes are seen in any part of the optic apparatus. The writer is inclined to interpret the results obtained in some of his lid sewing experiments (fig. 1-B) in the same manner as partial destruction of the retina or section of the optic nerve. The orbital suppuration following scarlet fever in Laura Bridgman might well account for the changes seen in her optic nerves and colliculi.

Attention is called especially to the full term dark room enucleated rabbit, as this is considered to be a most important experiment. In this animal the procedures of complete removal of peripheral sensory field and removal of sense organ were combined, and the results reported above show sharp contrast in the same brain.

It should be noted that the animal transferred to the light after five months and killed two months later showed no difference in any part of its optic system when compared with both the dark room and normal control rabbits. The fact that there was no additional growth or differentiation as a result of two months of peripheral stimulation would seem to indicate that full development was reached in spite of lack of use during the five months in the dark room. That this rabbit's lid and pupil reflexes were elicitable by the first flash of light ever to reach the retina appears to the writer to be proof that the retina and reflex centers were functional. The animal's rapid progress in its reactions to visual stimuli after removal to the light suggests that the entire system was functional and that only perceptual responses based on experience were lacking.

The present work supports Burr's summary concerning function not being a potent factor in the morphogenesis of the central nervous system. It also extends this thesis to include the post-natal development of the visual system in rabbits. Whether species other than the rabbit would give results similar to those reported here the writer is not prepared to say, nor can one predict what similar experiments on other sensory pathways might yield. The optic mechanism, being so highly specialized in function and differentiated in structure, seemed particularly favorable for study, and it was thought that if use were needed for structural development, the effects of its absence would be more striking in the optic system than in any other. The failure of such an outcome was a matter of some surprise.

It is apparent from the experiments reported in this paper that by having only one variable factor,—namely, the complete removal of peripheral stimuli without anatomical interference,—it has been possible to attribute the resulting changes, if any, to that variable and to it alone. It is not contended that peripheral sensory field plays no rôle whatsoever. It can be definitely stated, however, that the means of histological investigation and interpretation now at our disposal indicate that the lack of use has no effect on the optic pathways of the rabbit, and that the reflexes and

behavior of the animal removed to the light support the gross and cytological findings.

SUMMARY AND CONCLUSIONS

An anatomical-physiological study of the optic system of rabbits born and raised for six months in a completely dark room showed the following results:

1. There were no gross differences in any part of the eyes, optic nerves or tracts, primary optic centers, or occipital cortices.

2. Histological study of the retina in toluidin-blue, iron-hematoxylin, and Golgi silver preparations revealed no changes in thickness or regularity of layers, number or size of ganglion cells, character or amount of Nissl granules, or degree of cell process differentiation.

3. The optic nerves showed no changes in cross section area or myelinization.

4. The superior colliculi examined by the stains mentioned showed no changes in fiber or cell lamination, cell numbers, sizes, or types, nuclear size, Nissl substance, or amount of cell differentiation.

5. In like manner the lateral geniculates disclosed no alterations in the size or shape of the dorsal or ventral nuclei, the thickness or distribution of the optic tract, the number, size or types of cells, the nuclear size, Nissl bodies, or degree of cell differentiation.

6. The pulvinar presented no changes.

7. The striate cortex and cortical layers were not reduced in thickness; there were no changes in cell numbers, types, size or nuclear size, extranuclear chromatin, or cell differentiation.

One of the dark room animals with left eye extirpated on the ninth day after birth, and sacrificed at six months, showed in the same brain the difference between the two procedures of excision of an end organ and the complete removal of function. The significance of this particular experiment is pointed out in the text.

The lid and pupil reflexes of a dark room rabbit, removed to the light after five months for a study of its optic reflexes and behavior, responded immediately to the first stimulus of light. Within two weeks this animal showed excellent progress in its reactions to visual stimuli.

The following conclusion seems warranted: Complete lack of optic stimuli in rabbits from birth to six months of age results in no discernible changes in the visual pathways, does not prevent the lid and pupil reflexes from responding to the first stimulus of light or the animal from quickly learning to respond to visual stimuli in regulating its behavior; nor is there any indication that the presence of the peripheral sensory visual field is a factor in the development and differentiation of the optic system in rabbits, or in the ability of that system to function.

The writer wishes to thank Dr. George Burget for many helpful suggestions; and to express his deep indebtedness to Dr. Wm. F. Allen under whose direction this work was done, and whose constant advice and assistance have made these experiments possible.

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THE EFFECTS OF REPEATED ELECTRICAL STIMULATION OF THE CORTICAL MOTOR AREA IN THE CAT[1]

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Since much recent work has been done on the production of experimental convulsions by the use of convulsant drugs (e.g., absinth and camphor monobromide), I have thought that a return to the method of François-Franck in the production of clonic convulsions by electrical stimulation of the cortical motor area might serve to differentiate experimentally the direct effects of cortical excitation from the possible toxic effects of the convulsant drugs.

Cats were used in the experiments. After trephining the region over the cruciate fissure under ether anesthesia, platinum electrodes were inserted in the opening in the skull. Respiration and blood pressure were recorded. Ether was then intermitted and the experiment was begun as soon as the cortex became excitable electrically. Stimulation with a weak tetanic current was done for from two to five seconds every five minutes. The effects were those which have been recorded by François-Franck and many other observers,—an immediate clonic convulsion which persists from ten to thirty or more seconds after cessation of the stimulation. The tonic element is, however, less marked than in drug induced convulsions.

In a control series, stimulation was repeated at five minute intervals until the animals succumbed. In ten control animals, the minimal number of convulsions was thirty-seven and the maximum, fifty-two. The convulsions become less intense and of shorter duration as the experiment proceeds. Excitation of the opposite, previously unstimulated cortex at such a stage usually elicits a good clonic response except at the close of an experiment when the whole organism appears to be failing. Anatomical ablation of the region around one cruciate fissure and stimulation of the corresponding opposite side elicits clonic movements only contralateral to the intact side of the cortex.

Respiration is never so greatly accelerated as after the injection of convulsant drugs. Blood pressure falls gradually during the course of the

[1] The expenses of a part of this investigation were defrayed by a grant from the Commonwealth Fund to the Neurological Institute of New York.

experiment. Post-mortem examination usually showed a dilated stomach, general congestion of the abdominal viscera, and petechial hemorrhages in the lungs, as with drugs.

With the establishment of the general range of results in the control series, it becomes possible to use the method of electrical excitation of the cerebral cortex as a means of comparing the effects of cerebral anemia, paralysis of the skeletal musculature by curare and the administration of bromides upon the resistance of animals in which the convulsions were induced by toxic agents, such as absinth or camphor monobromide.

Cerebral anemia and cortical excitation. Anemia of the brain was induced by temporary ligation of the arteries to the head for varying periods of time and the effects of stimulation of the cortical motor area at different times were observed. After a control stimulation of the cortical motor area, with typical convulsive movements, the head arteries were occluded for from three to ten minutes and the cortical excitability was tested both during the period of occlusion and in the period of recovery.

In a series of ten animals it was found that stimulation of the cortex within one minute following the occlusion of the blood vessels produced a typical clonic convulsion; later in the occlusion period, the cortex was inexcitable. If the circulation was restored to the head within three minutes, and the general condition of the animal was good, it was usually possible to obtain a clonic convulsion on stimulation within ten minutes following the release of the head arteries. During the recovery period, stimulation of the cortical motor area caused a rise of blood pressure before the clonic convulsion could be elicited. The greatest number of occlusions combined with convulsions induced by electrical stimulation which could be obtained was six occlusions (2 to 3 minutes' duration) and ten clonic convulsions—considerably less than half the number of convulsions or occlusions obtained when done separately in control animals.

When the period of occlusion of the head arteries is longer (6 to 10 minutes) subsequent stimulation of the cortical motor area fails to produce any clonic movements at all within the time limits of an acute experiment. In acute experiments, under such conditions, tonic movements only may appear. The conditions after several days or weeks of recovery are yet to be studied.

Paralysis of the skeletal musculature by curare. It has been shown that a large part of the rise of blood pressure during the convulsive movements induced by drugs is due to the activity of the skeletal muscles (Coombs and Pike, 1931). Following the administration of absinth or camphor monobromide, the administration of curare produces a fall in blood pressure to a level from which there is no resuscitation. After a control excitation of the motor area, curare was injected intravenously and artificial respiration was maintained. Blood pressure was at first low but after about fif-

teen minutes it began to rise gradually. When it had risen to within 20 or 30 mm. of the control level, stimulation of the cortical motor area at the same rate and intensity as in the controls, was done. Although there was no observable movement of any muscle, blood pressure rose considerably more during stimulation of the motor area than in the control excitation.

In curarized animals, the number of excitations of the cortical motor area which could be done before the animals succumbed, was much greater than in the controls. The animals were still in good cardiovascular condition after 65 to 75 excitations of the cortex and the experiment usually had to be terminated either because of the exigency of the time, or the wearing off of the curare effect. In several animals in which this occurred, good clonic convulsions were obtained when the curare had ceased to be effective.

The effect of cortical excitation following administration of bromides. It has been shown that after the continuous administration of large doses of bromides for from two to three weeks, clonic convulsions do not appear after injection of absinth, but the animals succumb to lower doses of the convulsant drug than control animals (Pike and Notkin, 1931). Cats which had been fed 1.1 gram of sodium bromide daily for two to three weeks were prepared for stimulation of the cortical motor area. Using the same rate of stimulation and intensity of current which had been found to be effective in control animals, no clonic convulsions appeared in any of these cats. There were some movements of the limbs during excitation of the cortex, but these ceased as soon as stimulation ceased and were not clonic in type. Respiration became somewhat slower during the period of excitation but quickly regained its ordinary rhythm. The number of excitations possible under the conditions was greater than in the controls. After more than sixty excitations, the cats were still in good condition. At such times, when the lateness of the hour forced the end of an experiment, the intravenous injection of a low dose of absinth of camphor (0.02 cc. per lb.) caused the immediate death of the animal without a single convulsive movement. Blood pressure fell to zero and respiration ceased although the heart continued to beat for some time.

The post-mortem findings after electrial excitation as after injection of convulsant drugs are about the same—a rapid onset of rigor mortis (when there have been convulsions), dilatation of the stomach and colon, congested blood vessels of the viscera and petechial spots in the lungs being characteristic in both conditions.

Discussion. A comparison of the effects of convulsant drugs such as absinth or camphor monobromide with those of electrical excitation of the cerebral cortex brings out certain differences. First of all, the number of convulsions which the animal will withstand is from three to ten times as great after electrical excitation as after injection of convulsant drugs. Furthermore, the same conditions (e.g., curare or prolonged feeding of

bromides) which bring about an earlier demise of the animal after the injection of convulsant drugs may act to prolong the life of the animal when the cortex is excited electrically.

Although excitation of the cortical cells by whatever method elicits motor reactions through the autonomic as well as through the cerebro-spinal system, which are much the same whatever the method of excitation employed, other results seem to diverge sharply for some reason which we do not now know. These differences in the effects of the two types of convulsant agents, i.e., the physical and the chemical, must be considered in our speculations on the cause of epilepsy as it occurs clinically.

SUMMARY

1. Cats can withstand a much greater number of convulsions induced by electrical excitation of the cortical motor area than by convulsant drugs.

2. When the convulsions fail to occur because of blockage either in the cortical motor cells or at the myo-neural junction, the motor cortex can be stimulated almost indefinitely without causing the death of the animal.

3. When one side of the cortical motor area has been stimulated until there is no further bilateral clonic response, stimulation of the other side will still produce bilateral clonic convulsions.

4. After the first minute of cerebral anemia, electrical excitation of the cortical motor area is ineffective.

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AFFERENT IMPULSES AS A CAUSE OF INCREASED VENTILATION DURING MUSCULAR EXERCISE

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In the course of some observations on cardiac dyspnea, studies were made of the composition of the blood of normal men and of patients with cardiac disease before, during and after exercise (1). Except when the exertion was rather severe the changes in the acid-base state of the blood were inadequate to account for the increase in the ventilation which occurred quite regularly, even with very "mild" exercise. These findings are open to two possible interpretations: *a*, that the respiratory center is sensitive to changes in the blood which are too small to be detectable by the methods used, or *b*, that the increase in ventilation occurring under such conditions is due to some factors other than alterations in the acid-base condition of the blood.

The first of these two hypotheses seemed to us unlikely because in several instances the blood taken at the time when the ventilation was greatest was actually somewhat more alkaline than when the subject was at rest. Furthermore, additional experiments were done (2), and it was shown that dogs required a relatively large alteration in the acid-base state of the blood to produce changes in ventilation comparable to those occurring in man after slight exertion.

It seemed therefore that the explanation for the increased ventilation produced by mild exercise had to be sought elsewhere. The following experiments are the result of such an investigation.

RESULTS. A. *Observations on man.* Ventilation was measured either with a Benedict or with a Tissot spirometer. After the breathing had been recorded for several minutes in the resting state the subject was told to open and close the hands at a rapid rate. This exercise was performed for one minute. It was found that opening and closing the hands led to a slight increase in ventilation, and this occurred whether or not the circulation to and from the moving parts was impeded by a blood pressure cuff placed around the proximal portions of the arm and inflated to 200 mm. pressure. Such observations which are illustrated in figure 1, seemed to indicate that the increase in ventilation produced by this muscular effort could not be due to any chemical alteration in the blood induced by the muscular activity and suggested that the effect on respiration is due to "nervous" influence.

Theoretically, such a stimulation of breathing by means of the nervous system might be reflex, or it might be due to the irradiation of motor impulses from the cerebral cortex to the respiratory center (Krogh and Lindhard, 3). In figure 1 it is shown however that passive movements were likewise associated with increase in ventilation which occurred whether or not the circulation was intact. This finding suggests that the effect was of reflex origin and is in accord with the later observations of Krogh and Lindhard (4) in regard to passive movements.

Since the circulation of the bones was not obstructed it might be argued that the increase in ventilation was due to chemical changes in the blood passing to the general circulation by way of the bones. In order to settle this point further observations were made.

B. *Experiments on dogs.* The animals were anesthetized with barbital. Respiration was recorded in some instances, by allowing the dog to rebreathe oxygen from a Benedict spirometer. In other experiments, when accurate measurements of the ventilation were desired a different method

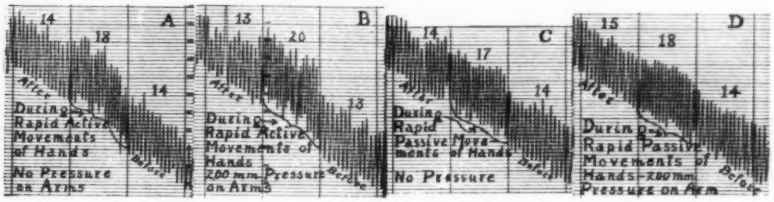

Fig. 1. The curve runs from right to left. The numbers refer to respirations per minute. The distance between the vertical lines represents one minute.

was used, the expired air being collected in a small spirometer. Passive movements were performed at a rate of about 300 per minute, an effort being made to avoid moving any parts of the body other than the extremity in question. This was not entirely possible in animals with intact extremities.

1. *The effect of cutting the spinal cord on the respiratory response to passive movements.* One animal showed a definite although slight response to movements of the hind-leg and of the fore-leg when the spinal cord was intact. After chordotomy, at the level of the sixth dorsal vertebra, the respiration was not affected by passive movements of the hind-leg but became accelerated when the fore-leg was moved.

In another animal (fig. 2) more striking results were obtained. When the spinal cord was intact well marked effects were obtained by moving, in order of increasing response, the tail, the hind-leg, the fore-leg and the head. (In the figure, for the sake of convenience in labelling, "leg" refers to the hind-leg and "arm" to the fore-leg.) It was noted that the

mass of muscle being moved was least when the tail was moved, more with the hind-leg, still more with the arm and most with the head. After the spinal cord had been cut at the fourth dorsal level passive movements of the fore-leg and of the head gave the same response as before, whereas movements of the hind-leg and of the tail were without effect.

It should be emphasized that such negative results were only obtained when the spinal cord was cut above the level of the nerve supply of the moving muscles. Thus, cutting the spinal cord at the ninth or tenth dorsal level did not always abolish the effect of moving the hind-leg because such movements were associated with slight movements of the abdominal muscles. However, cutting the spinal cord in the upper or middle dorsal region invariably abolished the respiratory response to movements of the hind-leg.

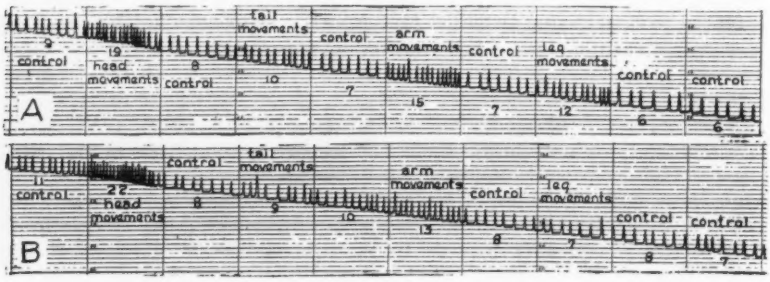

Fig. 2. The curve runs from right to left. The numbers refer to respirations per minute. The distance between the vertical lines represents one minute. A, record with spinal cord intact; B, record after spinal cord was cut at fourth dorsal level.

2. *The respiratory response to passive movements of legs completely amputated except for the femoral vessels and the sciatic nerve.* To the experiments on both men and dogs reported up to this point, two objections can be raised. In the first place since the bones were intact there is the possibility of the results having been influenced by blood returning from the moving extremity. In the second place, it seems very likely that the respiration was affected by motions of the muscles of the buttocks and of the abdomen. Although the data which have been presented point toward such an effect being reflex it might conceivably have been chemical as it was not possible when dealing with intact extremities to move them without causing some movements higher up and in no case was the venous return obstructed from these more proximal muscles.

In order to meet these objections the leg was amputated at the hip joint and all connections between the leg and the trunk except the femoral vessels and the sciatic nerve were severed. The femur was then tightly

clamped in a vise, in such a position that neither the vessels nor the nerves were under tension. This procedure had the double advantage of making it possible to isolate the chemical from the nervous effects and also allowed one to make vigorous passive movements of the leg without causing any movement in the muscles proximal to the leg.

The results are illustrated in figure 3. It was found that even when the the nerve and vessels were intact the effects of passive motions were rather slight, being usually much less than in the previous experiments. This is to be attributed to the shock which necessarily ensues following amputation, and also to the fact that the mass of moving vessels was less in the amputated than in the intact legs. Despite the smallness of the effect the increase in ventilation occurred in each experiment. (The maximum error of measurement with the small spirometers used is not greater than

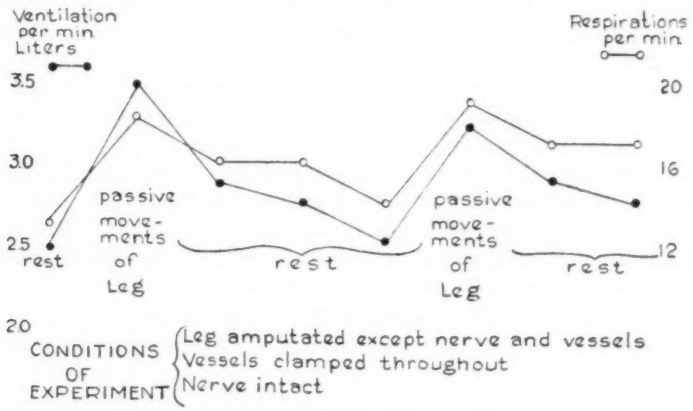

Fig. 3

0.04 liter and in no instance was the increase in ventilation on passive movements less than three times possible error.)

Following these observations the femoral vessels were occluded with bull dog clamps and the procedure was repeated. Again, in each of the experiments an increase in ventilation resulted, although in one of five animals this was scarcely greater than the error of measurement.

The clamps were then removed from the vessels, the sciatic nerve was cut, and after waiting a few minutes for the respiration to become constant, the procedure was repeated for the third time. In one experiment of the five a slight increase in ventilation resulted. In the remaining four instances moving the leg, when the sciatic nerve had been cut, was without effect even though the blood supply was intact.

3. *The rapidity of the response of the ventilation to passive movements.*

As can be seen in the figures the increase in breathing usually occurred immediately after the beginning of passive movements. This fact is probably the most convincing evidence obtained that the effect was of reflex rather than of chemical origin.

4. *The return of the ventilation to normal after the cessation of passive movements.* In instances in which the increase in ventilation was of small degree the respiration often returned to the previous control level as soon as the movements ceased. However, when a larger increase in ventilation occurred there was sometimes a "hang-over" effect, the respiration gradually returning to normal over a two or three minute period. This phenomenon was rather puzzling and led us to suspect at first that there might be a delayed chemical effect by means of alteration in the blood as well as an immediate reflex effect. However, experiments such as those portrayed in figure 3 showed that this was not the case. This animal showed a definite increase in ventilation for at least two minutes after the cessation of the movements. Since there was no possibility in this instance of any blood from the leg returning to the general circulation it is obvious that the delayed as well as the immediate increase in ventilation was due, in this instance at least, to nervous influences.

On second thought this did not seem surprising. It has been shown by Garrey (5) that stimulation of a nerve ganglion produces an increase in its metabolism, i.e., produces chemical changes in the ganglion. Hence, one might expect a delayed return of the respiration to normal for time might be required for reversal of the chemical changes induced by stimulation of the respiratory center, whether by reflexes or by other means.

SUMMARY. 1. Active movements of the extremities of man cause increased ventilation.

2. The result is not affected by the presence of an inflated blood pressure cuff proximal to the moving parts of the extremity.

3. Passive movements likewise increase the breathing of both man and dog.

4. Cutting the spinal cord of dogs abolishes the effect.

5. If the posterior extremity of a dog is severed from the body except for the femoral artery and vein and the sciatic nerve, movements of the extremity cause increase in ventilation regardless of obstruction of the blood vessels. Movements of the extremity are not associated with increase in ventilation if the sciatic nerve has been divided.

CONCLUSION

The increase in ventilation during muscular exercise, which is not severe enough to cause detectable alteration in the oxygenation or the acid-base condition of the blood, is due—in part at least—to respiratory reflexes arising in the moving parts.

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THE EFFECT OF METHYL, PROPYL AND BUTYL ALCOHOL ON THE GROWTH OF WHITE LEGHORN CHICKENS

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The aim of this investigation was to determine the effect of the administration of different concentrations of methyl, propyl and butyl alcohol on the growth and vigor of White Leghorn chickens.

METHODS. The alcohols were used in two concentrations, light and heavy. The initial concentration and dose were determined by experiments based on the fact that the anesthetizing power of the straight chain alcohols is proportional to their molecular weight. Various amounts of different concentrations of the alcohols were injected into the crops and the dosage chosen that brought on the primary symptoms of intoxication—staggering with occasional tumbles.

Ninety-five day old chicks were divided into groups as follows: 15 for each of the heavy alcohol groups, 14 for each of the light alcohol groups and 8 for controls which were given daily doses of water on the same schedule as that of the light alcohols.

The doses were administered each morning between six-thirty and eight o'clock by means of a graduated pipette, starting with a one cubic centimeter and ending with a twenty-five cubic centimeter pipette. These were inserted deep down into the esophagus, the liquid being released into the empty crop at a moderate rate of speed. If the pipettes were not inserted deep enough or the liquid allowed to run out too rapidly, the material would dam up in the esophagus and some of it enter the glottis causing irritation of the lining tissues and bringing about a condition of edema.

DOSAGE. The light methyl group received the following dosage: for the first 3 days, 0.25 cc. of 20 per cent; the next week, 0.5 cc.; the third week, 0.7 cc.; the fourth week, 0.7 cc. of 25 per cent which was gradually increased until at the end of two months they were receiving 2.5 cc. and at the end of 16 weeks most of them 12 cc. The heavy methyl group was treated as follows: for the first 3 days, 0.25 cc. of 40 per cent; for the next week, 0.3 cc. and the next, 0.4 cc.; this was gradually increased until at the end of two months it amounted to 1.5 cc. The concentration was then increased to 45 per cent and at the end of the third month they were

receiving 2.5 cc.; this was increased until at the end of the experiments the fowls were receiving from 5 to 9 cc. according to individual differences.

The initial dosage of heavy propyl alcohol was 0.25 cc. of 12 per cent, after 4 days 0.3 cc. was given, 6 days later 0.4 cc., at the end of 2 weeks 0.5 cc.; at the end of 3 weeks, 0.7 cc. At one month they were given 1 cc. of 13 per cent; at 2 months 2 cc. of 14 per cent; at 9 weeks 2.5 cc.; at 10 weeks, 2.5 cc. of 16 per cent; at 11 weeks, 3 cc.; at 12 weeks, 4 cc.; at 13 weeks, 4.5 cc. of 18 per cent. At 16 weeks they were receiving 6 cc. of 18 per cent. The light alcohol chicks were treated on the same schedule with one-half the concentration.

Butyl alcohol, with the greatest molecular weight, proved the most potent. The heavy alcohol group was treated as follows: for the first 3 days, 0.25 cc. of 6 per cent; for the next 4 days, 0.3 cc.; for the next 3 days, 0.4 cc.; at 2 weeks 0.6 cc.; at 18 days, 0.7 cc.; at 3 weeks, 0.8 cc.; at one month, 1 cc. of 7 per cent. At 10 weeks they were given 5 cc. of 8 per cent; this was gradually increased until at the end of the experiment they were receiving 7.5 cc. of 8 per cent alcohol. The light butyl group was injected on the same schedule with one-half the concentration.

Results. The growth of the four different lines is shown in table 1.

All the alcohols appeared to have a somewhat stimulating effect on growth during the first three weeks, but by the fourth week the heavy propyl group began to fall behind. They made consistently smaller gains than the other chicks and were considerably emaciated at the end of the experiment. At the age of 6 weeks the heavy methyl group began to lag, and at 9 weeks the same was true of the light propyl chicks.

If the sum of the average of the males and the average of the females in each group is divided by two we obtain the following final weights in grams: controls, 922; light methyl, 864; heavy butyl, 859; light butyl, 827; light propyl, 745; heavy methyl, 657; heavy butyl, 512. In other words, the two butyl and light methyl groups finished within 90 to 96 per cent of the weight of the controls; the light propyls at about 80 per cent, the heavy methyl at 71 per cent and the heavy propyl at 55 per cent.

The feathers of the chicks in the heavy methyl alcohol group were dull colored, ruffled and comparatively sparse; the muscles were flabby so that the wings generally dropped. The skin was pale and the combs small and anemic. These conditions were not so marked in the light alcohol group but were present to some degree. With five of the heavy methyl group abscesses formed in the wall of the crop. These plugs would fall out leaving a small hole which quickly healed. Macroscopic examination of the viscera of several of the birds failed to show any further abnormalities. These birds were nervous, especially the heavy alcohol

group. There was no evidence of the effects attributed to the drinking of this alcohol by man—blindness, deafness and paralysis.

There were more fatalities in the heavy propyl group than in the others, four of the chicks dying, because of the tendency of this drug to effervesce when injected into a crop which contained mash, causing an eruption of the material up the esophagus into the glottis and the consequent strangling of the chick. In this group the tails were sparsely feathered, the combs small, the feathers not compact nor sleek looking, while the skin and legs showed a tendency towards anemia. The same facts were true in regard

TABLE 1

Average weekly weight in grams

AGE	CONTROLS		LIGHT METHYL ALCOHOL		HEAVY METHYL ALCOHOL		LIGHT PROPYL ALCOHOL		HEAVY PROPYL ALCOHOL		LIGHT BUTYL ALCOHOL		HEAVY BUTYL ALCOHOL	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	Number of chicks at the end of the experiment													
	5	3	9	5	7	5	6	7	3	6	7	7	8	7
weeks														
0	39	39	36	35	35	36	37	35	36	35	36	36	37	36
1	53	50	47	47	56	50	47	57	50	48	47	53	61	52
2	71	70	73	81	89	75	73	79	76	66	76	86	83	80
3	103	105	107	124	138	109	119	121	111	102	120	128	121	132
4	143	140	142	160	178	142	159	154	144	126	158	192	172	167
5	191	193	184	220	207	175	200	188	178	162	192	206	204	201
6	271	261	263	263	231	194	261	239	229	209	262	272	263	251
7	324	295	312	325	297	223	337	297	298	243	321	346	349	341
8	385	354	369	358	354	273	377	315	338	265	377	388	383	374
9	447	421	427	413	414	313	407	350	341	277	435	450	433	421
10	518	488	483	468	458	332	481	396	367	314	480	497	511	479
11	562	515	523	513	480	349	536	442	401	334	528	537	576	530
12	626	565	581	541	583	387	598	479	422	358	574	582	616	574
13	720	652	676	597	622	402	665	507	436	383	627	643	692	648
14	827	700	766	663	744	443	700	541	488	415	679	700	766	707
15	909	752	841	719	761	472	757	592	511	441	756	744	848	735
16	1,012	832	959	768	841	472	868	622	553	471	856	798	932	786

to the light propyl group but to a less degree. There was a comparatively large number of hyper-irritable chicks in the propyl group, this being especially true among the heavy alcohol chicks.

Butyl alcohol affected the chicks the least of all, both as to weight and appearance. Almost the only difference between these birds and the controls was the anemic condition of most of the combs and the pale legs of the alcoholized chicks. On the whole this group was the tamest of all the fowls.

In a previous investigation (1930) light doses of ethyl alcohol had a

favorable effect on the growth and appearance of the chicks, this group outgrowing the other fowls and also having larger, redder combs and brighter, more compact feathers. The group receiving heavy doses of ethyl alcohol were more excitable than the others, while their feathers and combs were dull in color. The average weight attained at 16 weeks when males and females were averaged together as mentioned above was: light alcohol group, 982 grams, controls, 923 grams; heavy alcohol group, 856 grams.

SUMMARY

1. Heavy doses of methyl alcohol injected into the crops of growing chicks had an injurious effect on the growth and vigor as judged from appearance and also the disposition. Abscesses formed in the crop walls of a few of the subjects.

2. Light doses of methyl alcohol had a slight effect on growth, but influenced unfavorably the feathering, combs and disposition of the chicks.

3. Heavy doses of propyl alcohol had a markedly injurious effect on growth, and a somewhat deleterious one on appearance and disposition.

4. The same was true of light doses of propyl alcohol in a lesser degree.

5. Both light and heavy doses of butyl alcohol did not retard the growth of the chicks nor affect the appearance, except that the combs were somewhat anemic.

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EXTRAOCULAR REFLEXES

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The experiments reported here were instigated by the difference of opinion concerning receptors for the stretch reflex expressed in two papers appearing in 1928. The first by J. F. Fulton and J. PiSuñer, concluded that muscle spindles excite the reflex and suggested that tendon spindles probably inhibit it. The second, by D. Denny-Brown, assigned the excitatory rôle to the tendon receptors, the inhibitory to the muscle spindles. The extraocular muscles of the cat contain neither of these end organs (Cilimbaris, 1930) and hence should afford an instructive comparison with the extensors of the lower limb of the same animal studied in these two researches. Had we realized that P. Hoffmann (1929) had already reported the absence of the stretch reflex in extraocular muscles, or could we have anticipated the identification of the muscle spindle with the end organ responding to passive stretch by B. Matthews (1931), we should not not have undertaken the present work. Yet it seems worth reporting because it affords details of reflex patterns not previously described which bear upon the vexed question of the origin of the quick component of vestibular nystagmus.

METHOD. Cats were decerebrated under ether anesthesia, by the trephine method after inserting a tracheal cannula and looping the carotid arteries for temporary occlusion. Transection passed through or just above the superior colliculi and oral to the exit of the 3rd nerves. In case of transection between medulla and cord, this was done at the time of decerebration. The cartilaginous outer wall of one orbit was then excised; all extraocular muscles were identified by labelled silk ligatures, tied at their insertions, and freed from the globe, which was enucleated. In several instances a small patch of sclera was removed with each muscle to avoid injuring receptors at the insertion. Since such preparations showed no deviation in response, the practice was discontinued. The head holder was fixed firmly by a bar in a slot through the barrel of a microscope the base of which was screwed to a heavy block clamped to the table. In recording responses to stretch, the head was dropped by the coarse adjustment of the rachet and pinion of the microscope, the extent of drop recorded by one lever of the myograph attached to a drill hole in the bony orbit.

In the last two experiments a platform on which the cat was lying was moved in this manner, thus avoiding twist of the neck and hence the need of suboccipital transection. A frictionless double torsion lever recorded optically on the shadowgraph principle. For the last two experiments it was modified to a mirror principle. Early in the research the sensitivity was greatly enhanced by grinding down the torsion elements. After this modification the vibration frequency fell to 100 d.v. per second. Figure 1 shows records obtained prior to this change. With rare exceptions (fig. 2c and d) the recorded muscle was attached to the distal end of the lever shank by silk thread. Under these conditions, shortening approximates 1 mm. per gram. The tensions developed were so low that we do not believe the records were seriously distorted through stretch of the thread. This opinion is based chiefly upon the form of our records of submaximal neuromyal tetani some of which are shown in figure 2.

In recording from the recti muscles in reflexes such as the retraction associated with the protective wink, in which the entire retractor bulbi contracts powerfully, it is essential to distinguish between active contraction of the recti and their passive transmission of the pull from retractor bulbi. Our attempts to section the nerve supply as it enters a muscle without injury to the remainder of the nerve were unsuccessful. Instead, the retractor bulbi was tied out in isometric fixation. Of the three methods employed for this purpose, that which interfered least with the recording recti was to insert the thread from each muscle in a slit in a small wooden peg which was plugged into a hole in a brass bar fastened horizontally above the preparation. The pegs were then twisted, winding the threads to the desired tension in the manner of keys in tuning a stringed instrument.

Results. *Stretch.* The response to stretch was tested in twenty-three experiments in which controls were obtained after section of the motor nerve. Eleven of these were in animals with nervous system intact below the level of decerebration (six testing external rectus, two inferior oblique, three retractor bulbi). In one experiment on internal rectus, the only additional feature was suboccipital transection to eliminate neck reflexes. In eleven experiments (ten of them on external rectus) the labyrinths were silenced by either section of the eighth nerve or cocainization by the method of de Kleyn (Magnus, 1924). In six of them (five on external rectus, one on internal rectus,) the ipsilateral trigeminal nerve was cut. In nineteen experiments the change in tension was identical with that in controls after motor nerve section. In four, the tensions developed slightly exceeded those of the controls (fig. 1). Three of these were in external rectus, one in the superior external head of retractor bulbi. In none of these apparently positive experiments were reflexes from neck and labyrinth blocked. In several of the animals in which these possible sources of error were eliminated, permanent loss in tone of extraocular muscles was observed to follow

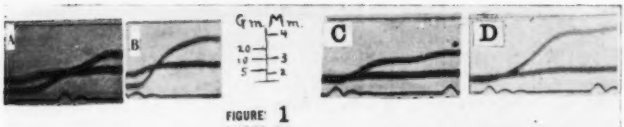
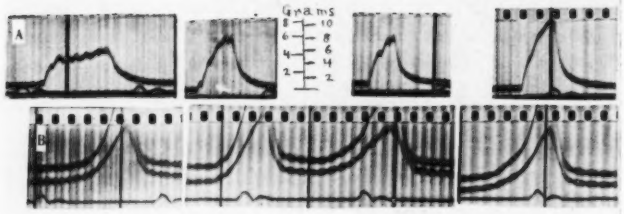


FIGURE 1



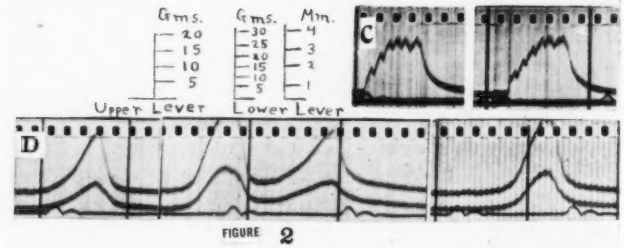


FIGURE 2

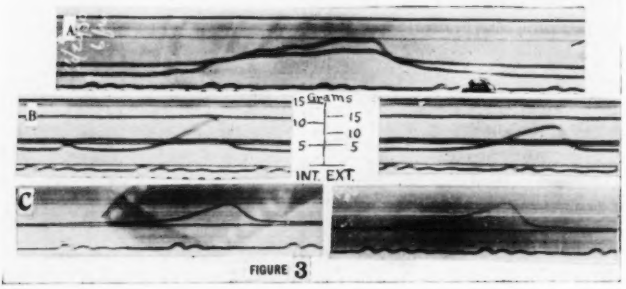


FIGURE 3

Fig. 1. Stretch of external rectus. Experiment of 1/25/29. A and B before, C and D after section of abducens nerve. The lever with the larger excursion indicates drop of the head. This is the greatest difference between experimental and control records obtained in the entire series. In all records lowest line indicates time in 0.2 second.

Fig. 2. A and C: Submaximal neuromyal tetani of internal rectus. Experiment of 5/8/29. B and D: Retraction reflex from stimulation of conjunctiva. Experiment of 4/24/29. Rectractor bulbi: Superior external head above; inferior internal head below. C and D are recorded more isometrically than A and B.

Fig. 3. Retraction reflex from stimulation of conjunctiva. A and B: External rectus above; internal rectus below. Experiment of 6/23/30. In A, the retractor is free; in B it is tied in isometric fixation. C: Gradual onset of recruitment in weak reflex. Experiment of 9/12/30. Inferior internal head of retractor bulbi. Standardization for external rectus in A and B is valid for C.

section of the eighth nerves or cocainization of the labyrinths. In no experiment could a jerk response be elicited by tapping the head holder.

Retraction reflex. In some of the experiments on stretch, occasional, brief contractions of considerable magnitude were observed which were concerned with retraction of the eye during the protective wink. These could be elicited in any preparation in good condition by touching the conjunctiva near the inner canthus. In some instances the conjunctiva of the other eye, or even the nasal mucosa proved an adequate field for eliciting the response. This reflex was studied in twenty-one experiments. No trace of it was observed in either superior or inferior oblique. All of the recti share in it passively, transmitting the pull of the strongly contracting retractor bulbi. The passive nature of this response was demonstrated in the case of superior and inferior rectus by the fact that it was not altered by section of the oculo-motor nerve. It was frequently abolished in all of the recti by isometric fixation of retractor bulbi. On the other hand, one or more of the recti often share actively in the contraction. Of the six experiments in which internal and external rectus were synchronously recorded, with fixation of retractor bulbi, internal rectus contracted in two, external in two, neither in two, though in all six both muscles showed the usual passive response with the retractor released (fig. 3). In one of the animals with active participation of internal rectus, it was noted that, prior to enucleation, the retraction was associated with internal rotation.

The form of the tension curve is invariably sigmoid. Recruitment is usually rapid (from 100 σ to 150 σ) and is often interrupted before attaining a plateau by relaxation almost as rapid and complete as that following a neuromyal tetanus (fig. 2). When the reflex is weak, however, it may recruit for as long as 300 σ, hold a plateau for 70 or 80 σ and relax at a declining rate for 200 σ to 300 σ. Such a sluggish wink may show three or four undulations in the course of recruitment (fig. 3). With isometric recording external rectus may develop a tension of 40 grams. With the considerable shortening permitted in most of our experiments, tensions of 10 to 20 grams are more common. Individual heads of retractor bulbi often show similar tensions; but in powerful retractions they may develop tensions which carry the lever shadow off the film (over 40 grams).

Our crude mechanical method of stimulation (touching the conjunctiva with a blunt muscle hook) does not permit determinations of true latency. Yet there can be no doubt that the duration of latent summation is extremely variable. Preparations in good condition responded to touch too light to give an artifact upon the record. Others in which it may be presumed the innervation of the conjunctiva was more damaged by the enucleation, often received strokes which pulled upon the tissue within the orbit sufficiently to induce a mechanical artifact. Such records indicate

that latent summation may continue as long as 240 σ before inducing a response and durations of 150 σ are common.

Nystagmus. In the experiments on stretch, the condition of the center was frequently tested by caloric vestibular nystagmus. "Spontaneous" nystagmus also occurred frequently in our records. Its vestibular origin is indicated by its permanent cessation after cocainization of the labyrinths.

TABLE 1

Influence of stretch on nystagmus

NUMBER OF BEATS MEASURED	EXPERIMENT OF 1/25/29	DURATION IN σ		SLOW / QUICK	TENSION			STRETCH
		Slow component	Quick component		Trough	Peak	Tensile change per beat	
					grams	*grams*	*grams*	*mm.*
7	Average	422	125	3.4	6.7	10.4	3.7	4.0
	Maximum	528	150	4.2	6.7	11.0	4.0	4.0
	Minimum	360	102	2.4	6.0	10.0	3.0	4.0
8	Average	379	137	2.9	4.0	6.4	2.4	0
	Maximum	460	150	3.5	4.0	7.0	3.0	0
	Minimum	312	120	2.2	4.0	6.0	2.0	0
	EXPERIMENT OF 4/3/29							
7	Average	348	242	1.4	1.0	3.0	2.0	0
	Maximum	378	276	1.7	1.0	3.0	2.0	0
	Minimum	330	222	1.3	1.0	3.0	2.0	0
6	Average	324	249	1.3	3.0	5.8	2.8	4.7
	Maximum	348	276	1.5	3.0	5.8	2.8	4.7
	Minimum	306	228	1.1	3.0	5.8	2.8	4.7
5	Average	343	269	1.3	1.5	3.5	2.0	1.0
	Maximum	372	288	1.5	1.5	3.5	2.0	1.0
	Minimum	324	252	1.2	1.5	3.5	2.0	1.0
7	Average	296	246	1.2	3.0	6.0	3.0	5.0
	Maximum	336	264	1.3	3.0	6.0	3.0	5.0
	Minimum	270	216	1.1	3.0	6.0	3.0	5.0

The pattern of this reflex contrasts with the retractor response in both distribution and course within the individual muscles. The change in tension per beat rarely exceeds four grams and is frequently less than one gram. Figure 4 shows a slow, strong, "spontaneous," lateral nystagmus. The myograms of internal and of external rectus are not mirror images of each other. Contraction develops by recruitment at an almost constant rate. Relaxation begins more abruptly, terminates more gradually. In strong,

rapid nystagmus contraction approaches more closely to a d'emblée response. Yet even here relaxation is more abrupt in onset. Duration ratios of $\frac{\text{slow}}{\text{quick}}$ components vary within a single experiment from 1.6 to 7.2. Sudden stretch or release from stretch by dropping or raising the head from one to five millimeters has no consistent observable effect upon the rhythm and no more upon the tensile change than it would induce in a neuromyal preparation (table 1). In one experiment (1/25/29) the slow

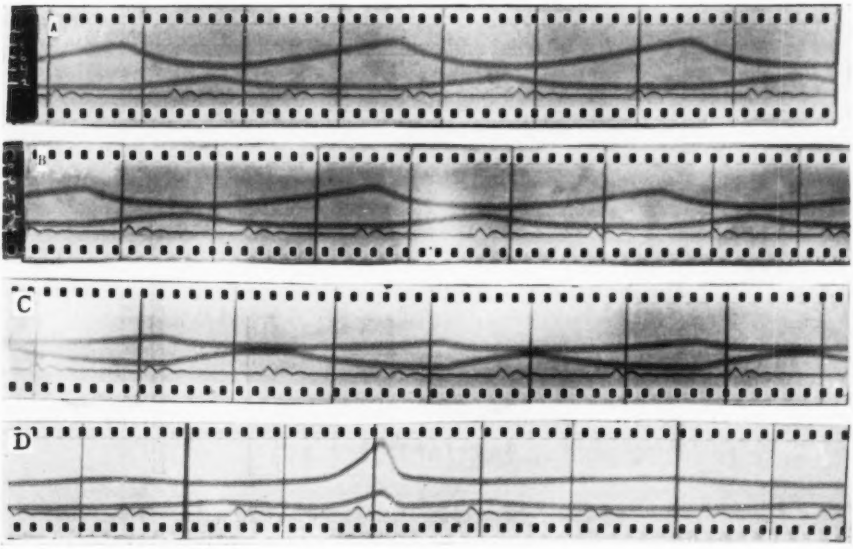


Fig. 4. Spontaneous nystagmus. A, B, and C: Experiment of 4/3/29. Internal rectus above; external rectus below. Standardization for tension of external rectus in A; for internal rectus in B. D: Experiment of 4/12/29. Retraction reflex from stimulation of nostril on a background of nystagmus. Internal rectus above; external rectus below. Retractor bulbi is free. The shortening in external rectus is probably due solely to tug from the origin of retractor, that in internal rectus chiefly to active contraction.

component averages slightly longer when stretched, in another (4/3/29 slightly shorter. The quick component is virtually unaffected. Active wink retraction in one muscle associated with probably passive pull in the antagonist leaves the nystagmus unchanged in rhythm or intensity (fig. 4 d). When nystagmus is induced calorically, the slow component is at first long, shortens as the intensity of the response increases, lengthens again as the reflex weakens. In marked contrast is the course of the quick

component, which may be constant within the limit of error of measurement throughout the entire period (fig. 5).

In two experiments an attempt was made to demonstrate nystagmus in retractor bulbi. Brisk lateral nystagmus was induced in the intact eye by irrigating the ear with cold water. With insertions of the recti and obliqui severed but the retractor untouched, irrigation induced visible minute movements of the eyeball. The eye was then enucleated but put back in the orbit. Retractor responses from stimulation of the conjunctiva still caused slight retraction of the eyeball. Nystagmus induced no perceptible movement. The eyeball was removed and the animal prepared for recording. In the internal and external recti nystagmus was vigorous. With all muscles free there appeared to be weak contraction of the retractor—so weak that we were unable to record it. The recti were then fixed

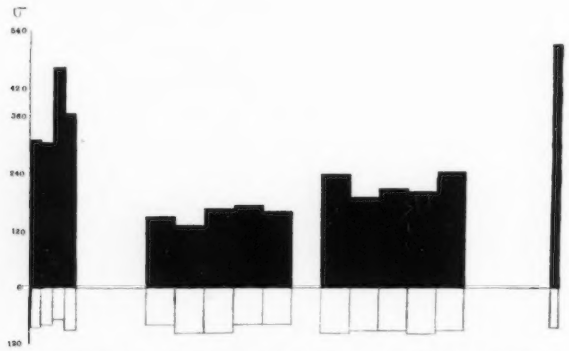


Fig. 5. Nystagmus from irrigation of ipsilateral ear with cold water. Experiment of 4/10/29. Duration of slow component plotted above the base line, that of quick component below it. Each upright represents a single beat. Thickness of uprights is proportional to change in tension. Gaps indicate periods in which the nystagmus is not plotted.

isometrically. The retractor became completely quiescent, nor could we induce in it any trace of response to irrigation while the recti were prevented from shortening.

Discussion. Reciprocal innervation was demonstrated for extra-ocular reflexes from the retina by Sherrington (1894) and for vestibular nystagmus by de Kleyn (1925). We know of no published records, however, obtained synchronously from antagonistic extraocular muscles under conditions sufficiently free from inertia to permit detailed analysis of their changes in tension or length. In the case of muscles of the extremities such a comparison is complicated by differences in distance of insertions from the moving joint and hence in effective tension and in degree of shortening.

In the case of the recti muscles of the eye the condition is almost perfectly symmetrical in length of muscle, direction of pull, and distance of insertion from the centre of rotation. We believe the levers we have employed permit an amount of shortening which approximates that occurring in the intact organism (about one millimeter per gram or about four millimeters in a vigorous nystagmus), and hence that our recorded tensions are of the order of magnitude of those which obtain in life.

If this be true for nystagmus, it cannot be true for the reflex of retraction. In this reflex the ratio of movement to tension in life is probably far smaller than that allowed by our levers when the muscles are attached to the distal holes on the lever shanks. For this reflex, therefore, our recorded tensions are probably considerably lower than those occurring in the intact animal.

In recording the retraction reflex in the recti, it proved essential to fix the retractor bulbi to avoid an artifact due to the pull at its origin upon the recording muscles. Dusser de Barenne and de Kleyn (1928), working with rabbits, have reported active participation of retractor in lateral nystagmus. The question arises whether such a response of the muscle exists in the cat, and, if so, whether it has complicated our records of nystagmus.

In view of the quiescence of retractor when the recti are prevented from shortening, we believe that in the cat the retractor does not share in nystagmus. We suspect the difference between our results and those of Dusser de Barenne and de Kleyn are due to a difference in reflex pattern between cat and rabbit. A difference in innervation of the retractor in the two species have been reported by Krause (1884) who states that in the rabbit the muscle is supplied by the oculo motor, in the cat by the abducens. It is of interest that Dusser de Barenne and de Kleyn report that section of the oculo motor abolishes nystagmus in this muscle but leaves its retractor function unimpaired. In the cat, some of our strongest retractor responses were obtained after section of the third nerve. G. S. Hopkins (1916) gives an interesting review of the conflicting literature on the innervation of this muscle. On the basis of dissections under a binocular microscope, he concludes that the innervation is solely from the abducens in cat, rabbit, and all other species he had examined. Clearly, it is unsafe to draw conclusions concerning the rôle of retractor in nystagmus in one species from data obtained upon another.

The origin of the quick component of nystagmus is still obscure. Bartels' theory, which invokes proprioceptors in the reacting extraocular muscles, has received scant credence. Yet the evidence against it is inconclusive. Bartels (1914) injected cocaine into the orbit in rabbits, and observed that both components were paralyzed. de Kleyn (1922) took advantage of the demonstration by Liljestrand and Magnus (1919) that small injections of novocaine abolish the tone of skeletal muscles due to their

own proprioceptors without impairing their exogenous reflexes. After enucleation, he injected the external rectus with varying doses of novocaine and found that with any dose which impaired nystagmus the slow component faded out pari passu with the quick. His conclusion that the proprioceptors must have been paralyzed before the disappearance of nystagmus rests upon no evidence of failure of proprioceptors in extraocular muscles, but solely upon the assumption of a vulnerability similar to that in the limb muscles injected by Liljestrand and Magnus. The work of Fulton and PiSuñer (1928), Denny-Brown (1928) and Matthews (1931) points to muscle or tendon spindles as responsible for the stretch reflex, which is presumably the factor eliminated by novocaine. In the rabbit, the extraocular muscles contain no spindles. Here the characteristic afferent endings are the terminaisons en grape (M. Hines, 1931). There is no direct evidence for the assumption that these endings or their afferents are more susceptible to novocaine than the motor nerves or endplates. Furthermore, the abrupt relaxations typical of the retraction reflex are strongly suggestive of the autogenous reflex inhibition implied in Bartels' theory. Further evidence would appear desirable.

Were the receptors of extraocular muscles the source of the quick component, the mechanism might be either a stretch reflex in the previously relaxing muscle which would inhibit the contraction in the antagonist, or a lengthening reaction in the previously contracting muscle which would excite a shortening reaction in the antagonist, or some combination of the two.

Our twenty-three experiments on stretch cover the entire range of magnitude and rate occurring in nystagmus. In only four did the tensions exceed the controls made after cutting the motor nerve. All four were in animals with reflex paths from neck and labyrinths intact. In all four the lowering of the head involved movement of the neck. In none could a jerk response be elicited by tapping the head holder. The difference in tension from that observed in the controls never exceeded that shown in figure 1. Therefore we regard them as instances of low grade contraction from exogenous reflexes (probably labyrinthine) and conclude that we have been unable to demonstrate the stretch reflex in extraocular muscles. In this we confirm the earlier results of P. Hoffmann (1929). Furthermore, the abrupt application or release of stretch during the course of a nystagmus has little if any effect upon the rhythm. Such changes as were observed do not exceed the limits of spontaneous variation, are inconstant in direction, and are far more pronounced in the slow than in the quick component (table 1). Active contraction, even when far exceeding the range occurring in lateral deflections of the eye, has no perceptible effect upon the rhythm of an intercurrent horizontal nystagmus. In figure 4 d a retraction reflex occurs upon this background. In this experiment there was no fixa-

tion of retractor bulbi. Comparison with experiments where this factor was controlled justifies, in our opinion, the conclusion that internal rectus (above) contracts powerfully, external rectus (below) passively transmits the pull from retractor bulbi. Neither event has a detectable influence upon the pattern or rhythm of the nystagmus. Hence we conclude in agreement with de Kleyn (1922) that it is highly improbable that either contraction or stretch of extraocular muscles determines the quick component of nystagmus.

Another theory worth considering suggests a labyrinthine origin for the quick as well as for the slow component. de Kleyn and Versteegh (1927) have induced post-rotation nystagmus in a rabbit in which one labyrinth was completely extirpated, the saccular and cochlear nerves cut and the ampulla of the posterior canal destroyed in the other. de Kleyn and Magnus (1921) have found nystagmus in the guinea pig after destruction of the otolith apparatus by centrifugalization. If the quick component be activated in the labyrinth, the most probable receptors would appear to be those of the ampulla which are responsible for the slow component also. Were this the case, any stimulation affecting the slow component would presumably affect the quick component also and to a commensurate degree. Hence the duration ratio of $\frac{\text{slow}}{\text{quick}}$ components should approximate a constant value, or, if deviations occur, they should bear a systematic relation to the intensity of the response. In a single experiment we find ratios ranging from 1.6 to 7.2. The low ratios include some of the lowest and some of the highest frequencies. We have found no relation between the relative duration of the two components and the intensity of the response. Figure 5 plots a response to irrigation of the ipsilateral ear with cold water. At the onset, the slow component is long, 460 σ to 300 σ. During the height of the response it drops to less than half these values. As the reaction subsides it stretches to durations of half a second. Throughout the entire period the quick component remains constant within the limit of error of measurement. In our opinion these data would be extremely difficult to reconcile with the assumption of an origin of the quick component from the receptors of the semicircular canals.

SUMMARY

Working on decerebrate cats we have been unable to demonstrate the stretch reflex in extraocular muscles.

The retraction reflex associated with the protective wink employs all four heads of retractor bulbi and often internal or external rectus in active contraction. The pull from these muscles is passively transmitted by the remaining recti through their close anatomical association at the ring of origin in the depth of the orbit. The reaction is one of rapid recruitment

to a tension which may exceed 40 grams but is frequently between 10 and 20 grams. The response is usually cut short before or shortly after attaining plateau level by relaxation which frequently approximates the speed of that which terminates a neuromyal tetanus.

In nystagmus the change in tension per beat rarely exceeds four grams and is frequently less than 1 gram. When the frequency is low, contraction recruits at an almost constant rate; relaxation in the antagonistic muscle begins more abruptly, ends more gradually. In rapid nystagmus contraction approximates a d'emblée response.

The rhythm of nystagmus is not disturbed by sudden application or release of passive stretch nor by the interpolation of a retraction reflex which contracts a muscle engaged in the nystagmus. These findings are opposed to Bartels' theory of origin of the quick component in proprioceptors of the extraocular muscles.

The duration ratio of $\frac{\text{slow}}{\text{quick}}$ component may vary from 1.6 to 7.2 in a single experiment. During the response to caloric stimulation the slow component is at first long, is reduced in duration by over 50 per cent during the height of the reaction, elongates again as it weakens. The duration of the quick component may be constant throughout the entire period of stimulation. These relations would be difficult to reconcile with a theory of origin of the quick component in the semicircular canals.

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PULSE WAVE VELOCITIES IN NINETY SUBJECTS[1]

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In reviewing the literature on pulse wave velocity we found reports based upon simultaneous subclavian-radial, carotid-radial, cardiogram-radial and brachial-radial tracings. None, however, presented the velocities in the same group of subjects for carotid-brachial-radial and subclavian-brachial-radial. Since many reports have been based upon carotid-radial tracings and as this method is indirect, it being necessary to subtract the innominate-carotid from the innominate-radial distance, it seemed desirable to compare the velocities thus determined with the subclavian-radial velocities in the same group of subjects.

In view of the finding of Bazett and Dreyer (1) that the velocity in small arteries is much greater than in large, it seemed very likely that the velocities found for subclavian-radial would differ from those for carotid-radial. At the same time we have made a number of additional observations, not all new, such as comparison of pulse wave velocity in men and women; confirmation of Bazett and Dreyer's finding; correlation of velocity with arterial blood pressure, systolic and diastolic, with heart rate and with other factors; effect of the respiratory arrhythmia and of auricular premature beats. We also have data upon family history of hypertension, height, weight and age. The study is of particular interest, however, in view of the recent report of Fulton and McSwiney (2) that there is no correlation between pulse wave velocity and arterial blood pressure in different individuals, since we find a significant correlation among women but not among men.

METHOD. The optical method was employed throughout. The source of light was a projection lantern. In order to obtain a sufficiently narrow band of light, a card with a vertical slit was placed before the lantern in the manner described by Wiggers (3). A Frank segment capsule was used for the subclavian or carotid artery, and for the brachial and radial small polysphygmograph tambours. The latter were more sensitive than the Frank capsule and proved more satisfactory for the smaller arteries. These

[1] Experimental work done in the Department of Physiology, Tulane University Medical School.

capsules or tambours were placed very carefully in a horizontal line, somewhat less than a meter from the slit of the string galvanometer camera. To one spoke of the wheel of the timer an extremely light strip of bamboo was glued. This strip cut through the three beams of reflected light from the tambour mirrors in such a manner that, as the wheel rotated, the three shadows thus formed fell upon the camera slit simultaneously. Parallax was thus avoided. The timer was checked for every two or three subjects against a Jacquet chronometer of known accuracy. The speed of the film was nearly always between the limits of 60 and 70 mm. per second, quite sufficient for accurate measurement.

To insure equal transmission times of the pressure changes from the receiving to the recording parts of the apparatus, equal lengths of small, fairly rigid rubber tubing were used. Tests showed no detectable difference in transmission times. The receiving parts, placed over the arteries, were of two types. For the carotid or subclavian, and for the brachial, shallow cups or capsules, fitted with outlet tubes, were cut from large corks. The open face of the one over the brachial was covered by a rubber membrane from which the center was cut. This capsule, when lightly taped over the artery in the cubital fossa, was rendered air-tight by the use of glycerin and undue pressure was thus avoided. Pressure somewhat greater than that used was found by test not to delay the pulse wave at this point. The most satisfactory method of recording the radial pulse was by means of a thin-walled, flexible bulb of a medicine dropper connected to the rubber tubing by a glass tube conveniently bent. This receiver was also taped in position.

In recording with the subject sitting at ease, the radial and brachial receivers were fixed in position and the receiving capsule for subclavian or carotid artery held firmly over the artery by hand. The simultaneous tracings of subclavian or carotid, brachial and radial made possible simultaneous determination of subclavian-brachial (or cartoid-brachial) and brachial-radial velocities.

To determine the length of the artery, the procedure was that of Bramwell, Hill and McSwiney (4), excepting that the distal measurement extended to the point on the radial over which the receiving bulb was placed.

Records were obtained from 90 subjects and in 14 of these tracings were made on two separate occasions. Forty-two of the subjects were women; 48, men. In 49 subjects both subclavian and carotid records were secured; in 40, subclavian only; in 1, carotid only. In a few instances, the brachial record was too poor for accurate measurement.

From each subject was also obtained a statement of age, height, weight, and any known family history of hypertension or cardiac disease. The arterial blood pressure, systolic and diastolic, was estimated by the auscultatory method both before and after recording the pulse waves. The heart

TABLE 1

SUBJECT NUMBER	SEX	AGE	BLOOD PRESSURE	HEART RATE (SUBCLAVIAN)	S-R VELOCITY	AVERAGE S-R VELOCITY	HEART RATE (CAROTID)	C-R VELOCITY	SUBJECT NUMBER	SEX	AGE	BLOOD PRESSURE	HEART RATE (SUBCLAVIAN)	S-R VELOCITY	AVERAGE S-R VELOCITY	HEART RATE (CAROTID)	C-R VELOCITY
1	M.	40	110–76	59	7.40	6.90			37	M.	23	124–80	70	8.90	12.90		
			118–82	75	8.55	10.30			38	M.	21	126–84	73	7.80	14.70		
6	M.	21	138–90	83	7.45	14.50	84	8.30	39	M.	20	104–70	88	7.60	12.80		
			140–90	100	7.25	12.10			40	M.	24	123–78*	71	7.95	13.90		
11	M.	22	112–77	91	7.00	11.10			41	F.	22	106–75	70	7.20	9.00		
			115–81	80	6.30	12.80			42	F.	23	96–63	86	6.75	9.80	78	7.40
12	F.	26	102–73	92	6.30	6.80			43	F.	22	123–82	80	7.20	11.05	76	7.40
			102–65	86	6.40	8.40			44	F.	22	96–57	62	5.45	10.55	69	6.20
14	M.	31	120–66	59	7.00	11.80			45	F.	30	101–73	75	8.30	11.30	77	9.10
			113–75	79	8.00	10.50	84	8.75	46	F.	21	112–77	91	7.30	8.85	92	8.50
17	M.	27	107–78	76	8.25	10.30			47	M.	24	114–85	78	8.00		83	9.20
			106–70	90	8.20	12.50			48	F.	20	108–74*	86	6.85	9.40	86	7.30
19	M.	23	112–78	65	6.40	9.00			50	F.	19	118–78*	101	7.90	12.70	107	10.20
			118–78	57	6.70				51	M.	25	117–72	75	7.70	9.70	82	10.30
21	F.	29	110–84*	71	8.90	11.20			52	F.	17	105–73	82	7.50	16.30	87	7.60
			112–85	72	11.00	11.90			53	M.	20	115–82	63	7.50	12.60	66	8.50
25	M.	37	138–86	121	8.25	9.90			54	F.	31	128–79*	94	8.00	10.75	97	9.30
			115–79	92	7.60	8.40			55	F.	36	128–82*	64	8.70	11.80		
27	F.	24	110–76*	83	7.65	9.90			56	M.	20	120–74*	48	6.70	10.10	59	7.15
			106–74	78	7.70	9.90	76	8.90	57	M.	21	103–75	65	7.35		70	7.75
31	M.	31	112–82	93	8.15	13.90			58	M.	44	110–65	68	7.30	9.40	70	7.40
			113–79	92	8.70	12.30			59	F.	21	103–63	63	6.40	9.20	69	6.95
33	F.	22	120–90	58	8.60	10.90			60	F.	22	112–78*	74	5.80		80	7.25
			111–83	73	8.00	9.75			61	M.	27	117–86	76	7.70	14.55	75	8.55
49	F.	20	113–72	122	7.50	9.30	121	8.40	62	M.	22	140–86*	64	6.95	10.25	64	8.70
			104–70	89	6.80	8.40	100	7.15	63	M.	24	117–84	79	8.45	11.60	92	9.35
80	F.	24	111–75	113	8.55	10.00			64	M.	22	118–85			11.80	68	7.95
			108–76	91	6.15	7.10	92	6.80	65	M.	22	108–85	73	7.25	7.30		
2	F.	33	102–74	77	6.70	9.05			66	F.	18	112–74*	84	7.50	10.70	85	8.20
3	M.	22	96–68	59	7.30	12.50			67	F.	19	96–64	86	6.15	9.75	91	6.50
4	M.	22	112–78	75	6.50	11.80			68	F.	21	104–72	98	7.60	11.20	94	8.80
5	M.	23	125–82*	95	7.40	9.85			69	F.	17	102–68	84	7.20	8.80	87	7.60
7	M.	25	112–74	99	6.85	10.30			70	F.	19	128–88*	89	7.90	11.75	92	8.50
8	M.	22	105–75	77	7.10	9.95			71	F.	22	106–73	88	6.90	9.20	91	7.60
9	F.	23	84–64	66	6.00	8.05			72	M.	24	122–84	44	7.20	13.70	50	8.20
10	F.	32	106–76*	90	7.20	8.80			73	F.	19	122–78	88	8.40	11.35	94	9.40
13	F.	25	105–78	91	7.90	11.20			74	F.	21	106–72	93	7.10	11.40	86	7.80
15	M.	21	107–80	70	6.00	6.45			75	F.	25	128–76	82	6.70	9.30	90	6.85
16	M.	20	104–76	76	7.05	10.50			76	F.	23	106–70	68	6.45	8.80		
18	M.	26	109–78	80	7.30	12.60			77	F.	22	138–94	91	7.60	10.60	92	8.05
20	F.	22	110–84	70	8.60	8.50			78	F.	20	116–76	72	6.95	11.15	78	7.80
22	M.	21	111–65	77	7.05	10.90			79	F.	24	108–74	78	5.85	8.20	88	6.45
23	M.	22	107–82	76	7.45	11.10			81	M.	20	114–80	80	7.20	10.60	83	7.85
24	M.	27	110–72	89	6.40	11.00			82	M.	21	120–86	85	6.45	11.65	94	7.30

TABLE 1—*Concluded*

SUBJECT NUMBER	SEX	AGE	BLOOD PRESSURE	HEART RATE (SUBCLAVIAN)	S-R VELOCITY	AVERAGE S-R VELOCITY	HEART RATE (CAROTID)	C-R VELOCITY	SUBJECT NUMBER	SEX	AGE	BLOOD PRESSURE	HEART RATE (SUBCLAVIAN)	S-R VELOCITY	AVERAGE S-R VELOCITY	HEART RATE (CAROTID)	C-R VELOCITY
26	M.	35	114–82	83	8.50	10.10			83	F.	21	103–73*	79	7.50	10.95	82	7.85
28	M.	26	150–85*	86	8.00	9.75			84	M.	22	116–80	89	8.85		96	10.15
29	M.	21	112–75	78	7.05	10.60			85	M.	21	120–77	60	6.60	8.65	68	7.00
30	M.	23	118–75	73	6.30	10.90			86	F.	23	109–64	66	5.75	5.70	74	5.80
32	M.	29	111–79	92	8.30	12.30			87	M.	19	140–88	72	6.50	12.15	72	7.75
34	F.	20	98–58	72	5.55	6.90			88	M.	20	130–80	66	6.35		67	7.30
35	F.	18	106–65	71	6.40	9.90			89	M.	22	122–68	62	7.00	13.70	62	8.20
36	F.	28	107–78	91	9.20	11.40			90	M.	21	120–75	56	7.00	12.20	64	8.70

* Indicates family history of hypertension.

rate was determined directly from that portion of the tracing on which the other measurements were made.

Time intervals between pulse waves were measured by reference to the most convenient time line. An accurate millimeter ruler and hand lens were used for this purpose. To fix the beginning of the up-stroke of each wave, a mark was first made with a fine-pointed needle. Generally the time intervals used in calculation are averages of individual measurements of not fewer than ten different beats. Many of the tracings were measured independently by each of us and the agreement was remarkably close.

RESULTS AND DISCUSSION. 1. *Pulse wave velocity range and average.* The carotid-radial velocity averages 8.007 $\pm$ 0.098 meters per second in 49 subjects, with a range of from 5.80 to 10.30. This is a considerably higher velocity than that reported by Bramwell, Hill and McSwiney (4) for comparable ages, namely, 6.45 meters per second. Some of the reasons for the higher velocity in our cases are apparent. In the experiments of the above authors, the radial pulse was recorded from a cuff, 8 cm. wide, around the forearm. Thus the 8 cm. had to be subtracted from the total length of artery for their calculation. Since the highest velocities are in the smallest arteries, their procedure will necessarily give a velocity lower than that obtaining for the full carotid-radial distance. Calculation from our own data shows, however, that this factor probably does not account for more than about 25 per cent of the discrepancy. Thus, if this factor were allowed for, our mean velocity would still be about 7.65 meters per second. Another factor is perhaps the more complete relaxation of Bramwell, Hill and McSwiney's subjects. It is also not impossible that the method employed by them for recording the radial pulsation produced a delay as compared with that used by us. If so, this would tend to make their velocities too low.

The average subclavian-radial velocity on the same group of 49 subjects was 7.163 ± 0.071 meters per second, with a range of from 5.45 to 8.85. This average is 0.844 ± 0.121 meter per second slower than the C-R (carotid-radial) velocity already given. In view of the magnitude of the difference and of its probable error, there can be no doubt that any large group of subjects would present a considerable difference in C-R and S-R (subclavian-radial) velocities. Individually there was a fairly wide range in this difference, i.e., from 0.05 to 2.60 meters, but in no case was the S-R velocity greater than the C-R.

The reason for the difference in these velocities is not difficult to understand. The chief factor was undoubtedly the size of the artery. In calculating the C-R velocity, the arterial length used was determined by subtracting the innominate-carotid distance from the innominate-radial. Since the common carotid is large, the effect is equivalent to subtracting a part of this length of large artery from the subclavian-radial distance. The difference in the two velocities, therefore, is added confirmation of Bazett and Dreyer's observation that the pulse wave is propagated more rapidly in small than in large arteries.

Assuming that the pulse wave velocity in the subclavian-axillary is, on the average, equal to that in the common carotid, we may make an indirect calculation of the velocity in the latter. The velocity calculated is 3.15 meters per second. The mean diastolic blood pressure was about 76 mm. of mercury. If we allow about 16 mm. for the difference in hydrostatic pressure between the carotid and brachial in the sitting position, we may estimate the mean carotid diastolic pressure at about 60 mm. Hg. The pulse wave velocity in isolated carotid arteries from the younger individuals in Bramwell, Downing and Hill's (5) experiments was about 5 meters per second at this pressure (their fig. 3). Bramwell, McDowall and McSwiney (6) reported an average velocity of approximately 4.5 meters per second at a pressure of 60 mm. in the artery *in situ*; but in their experiments a smaller artery, apparently the brachial, was used. In an earlier paper, at a pressure of 57 mm. in the isolated carotid artery of a young man, Bramwell and Hill (7) found a velocity of 3.45 meters. Bazett and Dreyer estimated the velocity from heart to carotid at about 3 to 4 meters at a diastolic pressure of 70 to 75 mm. Therefore the velocity of 3.15 which we calculate for the common carotid is in fair agreement with the findings of other authors, although the method of indirect calculation involves an assumption which may not be true.

There was a group of only 43 subjects in whom both carotid-brachial and subclavian-brachial velocities are available. In these, in contrast to an average C-R pulse wave velocity in the same group of 7.98 meters per second, the mean C-B (carotid-brachial) velocity is 6.83. This latter velocity is lower, of course, because of the average larger size of the vessel.

In the same way the average subclavian-brachial velocity is but 6.01 meters per second in the same 43 subjects in comparison with an average S-R velocity of 7.15. The difference between S-R and C-R is 0.83 meter, and between S-B and C-B, 0.82.

The velocity in the radial artery, in agreement with the finding of Bazett and Dreyer, is considerably higher than that in the other arteries. Our mean brachial-radial velocity, from 98 determinations, is 10.60 ± 0.131 meters per second, with individual variations from 5.70 to 16.30. Bazett and Dreyer state that the average velocity from elbow to wrist is 8.5 meters per second, although an average B-R velocity of the ten normal cases given in their tables is exactly 10.00 meters. This is very close to our average.

According to Moen's (8) formula, the factors, aside from pressure, which may influence the velocity are the elasticity and thickness of the vessel wall and the diameter of its lumen. As Bazett and Dreyer suggest, it is these factors, particularly the diameter of the vessel, which account for the higher velocity in the radial artery. As Sands (9) suggests, no doubt various influences, among which the nervous is probably the more important, may very considerably modify the pulse wave velocity, particularly in the radial.

In recapitulation, our evidence shows that the velocity of the pulse wave in general increases in the following vessels in the order named: subclavian-axillary and carotid, brachial, radial.

2. *Diastolic pressure and pulse wave velocity.* There is every reason to believe that the factor of primary importance in determining pulse wave velocity in a given vessel is the diastolic blood pressure (6), (7). With this in mind, we have compared the velocities, not only from the subclavian or carotid to radial, but also from the subclavian or carotid to brachial and from the brachial to radial, with the diastolic pressures. If the table be examined, it will be seen that there is a general correspondence between the two, but that there are numerous individual exceptions. To a large extent, no doubt, the exceptions are to be attributed to other factors, such as elasticity of the arterial wall or average caliber of the lumen. In calculating the coefficients of correlation, we have included in each case the two readings from each subject upon whom the determination was repeated. Such inclusion, while not an orthodox statistical procedure, affects the results hardly at all. The average or mean S-R velocity in 102 determinations (including 13 repetitions) is 7.33 ± 0.06 meters per second, with a mean diastolic pressure of 76.5 ± 0.47 mm. Hg. The standard deviations were respectively 0.8935 ± 0.0423 and 7.020 ± 0.337. The correlation coefficient was 0.496 ± 0.050. Since the probable error is only about a tenth of the coefficient, the correlation is certainly significant. A

coefficient of correlation of this magnitude means that there is about a 67 per cent association between the pressure and the velocity (10).

In comparison with these figures, in 96 determinations where the readings were available, the average subclavian-brachial velocity was 6.26 ± 0.066 meters per second, with a mean diastolic pressure of 76.77 ± 0.515 mm. Hg, and a coefficient of correlation of 0.384 ± 0.059. Here the association is less close, but it is, nevertheless, significant.

Brachial-radial velocities were available from 98 determinations. Here the mean velocity was distinctly higher, 10.60 ± 0.131 meters per second, while the mean diastolic pressure was 76.99 ± 0.515 mm. Hg. The coefficient of correlation was 0.328 ± 0.061, again significant, but with a percentage of association of only about 61. It is to be expected that other factors than pressure, such as vessel caliber, will be a more important influence in small than in large arteries. A further calculation shows that there is no significant correlation between B-R and S-B velocities. This would appear to mean that many cases which showed a correspondence between diastolic pressure and the one velocity gave none for the other.

Turning to our carotid measurements on 49 subjects, the mean C-R velocity was 8.025 ± 0.103 meters (by indirect calculation, compared with the direct given above); the mean diastolic pressure 76.174 ± 0.759 mm. Hg; and the correlation coefficient 0.395 ± 0.083. In comparison with these figures, the S-R velocity in the same 49 subjects was 7.199 ± 0.077, and the coefficient of correlation with diastolic pressure, 0.336 ± 0.086. It appears, therefore, that C-R and S-R pulse wave velocities correlate about equally well with diastolic pressure.

3. *Systolic blood pressure and pulse wave velocity.* The coefficient of correlation for these was 0.246 ± 0.065. This does not mean that the velocity is in any way directly influenced by systolic pressure, but, as we interpret it, that in general a high systolic means a relatively high diastolic pressure and vice versa.

There was no significant correlation between pulse pressure and pulse wave velocity, the calculated coefficient being −0.100 ±0.067.

4. *Sex differences.* The average heart rate in 55 determinations on 47 male subjects was 77.09 ± 1.27 beats per minute, and the diastolic pressure, 79.32 ± 0.555 mm. Hg. In 47 determinations on 42 female subjects the figures were 81.91 ± 1.26 and 74.21 ± 0.763 respectively. Along with these differences in heart rate and diastolic pressure, there were corresponding differences in mean S-R pulse wave velocity; the figures for men and women being 7.423 ± 0.067 and 7.239 ± 0.097 respectively, a percentage difference of 2.5. The B-R velocities were distinctly higher in the men, 11.26 ± 0.191 against 9.947 ± 0.175 for the women, a percentage difference

of 13.2. These differences are probably to be explained on the basis of the difference in diastolic pressures in the two groups.

When we separate the sexes and correlate the pulse wave velocities with the diastolic blood pressures, unexpected differences appear. In the 47 determinations on women the correlation (S-R and diastolic pressure) was 0.662 ± 0.0565, a percentage of association of nearly 74. In the 55 determinations on men the correlation was 0.0934 ± 0.091. Among the women, therefore, the correlation is fairly close, while in men it is absent. The same difference emerges when we correlate carotid-radial velocities and diastolic pressures; for 29 women, 0.529 ± 0.093; for 20 men, 0.146 ± 0.156. The similar correlations using brachial-radial velocities show, for 47 determinations on women, 0.326 ± 0.090 and for 50 determinations on men, 0.0813 ± 0.0935.

The discovery of this difference led us to wonder whether accurate estimation of diastolic pressure is more difficult in the more muscular arms of men than of women. That the contrast is not due to such an error is clearly shown, however, by a separate correlation in the two sexes between systolic pressures and velocities. The same sex difference again appears in quite unambiguous fashion, the coefficients being 0.550 ± 0.0702 and 0.057 ± 0.0895 respectively for women and men. Dr. James A. Bradley suggested to us that a greater variation in arterial caliber in men than in women, due to greater differences in muscular development in the former, may explain this remarkable and, at least statistically, significant sex difference. In this connection it is of interest to note that Fulton and McSwiney (2) report no correlation between blood pressure and pulse wave velocity in different subjects. Although the authors do not so state, it may be that their subjects were chiefly male.

The correlation between heart rate and S-R velocity in 55 determinations on 47 men is 0.329 ± 0.081; in 47 determinations on 42 women, 0.172 ± 0.0975. In neither case is the percentage association great, although there appears to be a slight relation between rate and velocity, probably dependent on the fact there tends to be, *ceteris paribus*, an increase in pressure with a rise in rate. The difference here shown between men and women is probably not statistically significant.

5. *Heart rate and diastolic blood pressure.* Correlating the heart rate and diastolic pressure in 103 determinations on 89 subjects, we obtain a coefficient of 0.0915 ± 0.066, not a statistically significant relation. However, when the heart rates of the two sexes are adjusted to a common basis and the correlation then made, the coefficient becomes 0.167 ± 0.065, that is, nearly three times its probable error. We may, therefore, say that, other things being equal, there is probably some relation between the two, but other factors preclude a significant correlation.

6. *Heart rate and pulse wave velocity.* Here again the correlation

coefficient, 0.250 ± 0.066, while higher than that relating rate and diastolic pressure, is not high. When adjustment is made for the sex differences in heart rate the coefficient rises slightly to 0.277 ± 0.062.

7. *Variations depending upon physiological state of the individual.* In case of several of the subjects upon whom duplicate determinations were made at different times, striking differences in pulse wave velocity were recorded. For convenience these have all been placed at the head of the accompanying table. It will be observed that there is a change in the velocities when both heart rate and diastolic pressure vary considerably. This observation serves to emphasize the necessity of complete relaxation of the subject if what might be called the basal pulse wave velocity is to be determined. On one occasion, not shown in the table, we attempted to determine the change in velocity in one arm while the opposite arm was immersed in ice water. The increase in S-R velocity was from 6.21 to 7.05 meters, but it is impossible to tell whether it followed a rise in pressure due to vasoconstriction produced by cold or by discomfort. Observation demonstrated a rise in diastolic pressure with immersion of the arm. Sands' (9) experiments in which dry heat applied to the arm was found to decrease the velocity are less open to this criticism.

Vigorous exercise, tried on one occasion, increased the carotid-radial velocity from an average of 7.65 to 8.74 meters per second.

Frequent auricular premature beats, observed in one case, produced a change in subclavian-radial velocity which was hardly greater than the probable error in the measurements. For the beats terminating the longer cycles following the premature beats the average velocity was 6.04 meters; for beats terminating cycles of average length, the velocity was 6.16 meters; and the pulse wave of the premature beats travelled 6.36 meters per second. While it may be questioned whether a real increase is demonstrated, as would be expected on theory, we are confident that the velocity of the premature waves is not less than that of the normal waves as maintained by Laubry, Mougeot and Giroux (11), (12).

Partial confirmation of the above finding is derived from a study of a case of sinus arrhythmia and bradycardia. Here the effect was unquestionable. During the sinus arrhythmia the average subclavian-radial velocity for seven beats terminating cycles of 1.2 seconds was 6.80; for seven beats ending cycles of 0.98 second the average velocity was 7.56; and for 17 beats following cycles of intermediate length, 7.20 meters. These changes are in agreement with those reported by Hickson and McSwiney (13).

Holding the breath to the breaking point was another influence causing a rise in the velocity (S-R), from 8.22 to 9.67 meters per second.

8. No significant relationship was found between pulse wave velocity and family history of hypertension.

It is important to note that in all correlations the exceptionally high

S-R velocity of 11 meters found on one occasion in subject number 21 was omitted.

SUMMARY

The average carotid-radial pulse wave velocity in 49 subjects was 8.007 meters per second; the average subclavian-radial, in 102 determinations on 89 subjects, 7.33 meters. The average velocity from carotid to brachial was 6.83, and from subclavian to brachial, 6.26. From brachial to radial it was 10.60. The velocity in the carotid artery is indirectly calculated to have been 3.15 meters per second.

There is a slight positive correlation between pulse wave velocity and heart rate.

The correlation between pulse wave velocity and systolic or diastolic blood pressure is good in women. In men both these correlations fail.

In a single case the pulse wave of auricular premature beats was found to travel, not more slowly, but if anything more rapidly than the other waves.

No significant difference was found between the groups who did and who did not report family history of hypertension.

The physiological state of the individual exerts a marked influence upon the pulse wave velocity, as detailed in the body of the paper.

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THE RESERVOIR FUNCTION OF THE SPLEEN IN FOWLS

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The discovery by Barcroft of the reservoir function of the spleen in mammals has raised the question whether this function exists in other groups of animals, and if so, whether it is subserved by the spleen. We selected the domestic fowl as a representative of a group, the birds, in which we do not believe this question has been studied.

METHODS. Single comb leghorns were used and blood obtained from a needle stab into a vein in the wing. Hemoglobin estimations were made on these samples by means of a Dare hemoglobinometer. The bird was put in a small coop in the dark for about an hour before the "resting" estimations were made and the samples taken with a minimum of scaring and stimulation.

Immediately after the "resting" estimations had been made asphyxia was induced by pinching the trachea from outside until the bird went limp. Then a sample from the opposite wing was taken as quickly as possible for the "asphyxiated" estimation.

Each figure quoted is the mathematical average of ten readings on the same sample. The standard deviation and mean error of these readings were computed and only those differences are quoted as significant in which the difference between the means of the two sets of readings is greater than four times the probable error of the difference.

The splenectomy operation was made as follows: Fifty milligrams of amytal per kilo were injected into the body cavity and the anesthesia was completed by ether inhalation.

A skin incision two inches long was made just ventral and caudal to the right wing. By blunt dissection through the peritoneal folds slightly to the right of the gall bladder, the spleen was exposed. After rupture of its capsule the organ was enucleated with the finger and bleeding checked by tight packing with dry cotton which was left in place for five minutes. After removal of the cotton the skin and body wall were closed as two separate layers with interrupted thread sutures.

Apart from ordinary cleanliness no aseptic precautions were found to be necessary. Three or four days after the operation the hens appeared

to be perfectly normal. The wounds healed by first intention and were almost invisible by the end of six weeks, which was the time arbitrarily allowed for complete recovery before further hemoglobin estimations were made—in one case the hen was already laying again.

The control operation was identical with that just described except that the spleen after exposure and identification was left intact.

The total operative and anesthetic mortality was a little over 60 per cent.

TABLE 1

The effect of partial asphyxia on the hemoglobin number of normal and splenectomized hens

HEN NO.	BEFORE SPLENECTOMY			AFTER SPLENECTOMY		
	Before asphyxia	After asphyxia	Difference in per cent of normal	Before asphyxia	After asphyxia	Difference in per cent of normal
4237	63	66	+5	63	63	0
4427	58	66	+14	63	63	0
B346	40	43	+8	62	62	0
D777*	32*	39	+22	68	67	−1
D766*	31*	34	+10	39*	38	−3
D794*	35*	37	+6	50	50	0
D771*	30*	34	+13	52	52	0

* Those hens with a low hemoglobin number were all laying, whereas those with a high number were not. There seems to be some correlation between hemoglobin value and the egg laying cycle.

TABLE 2

The effect of partial asphyxia on the hemoglobin number of normal and operated but non-splenectomized hens

HEN NO.	BEFORE OPERATION			AFTER OPERATION		
	Before asphyxia	After asphyxia	Difference in per cent of normal	Before asphyxia	After asphyxia	Difference in per cent of normal
4293	62	72	+16	56	63	+13
5574	54	61	+13	54	66	+22
5564	64	78	+22	63	67	+6

RESULTS. Tables 1 and 2 show first that in normal hens asphyxia is accompanied by an increase in the hemoglobin content of the blood. Besides those figured in the table we obtained similar data for some fifteen other birds. We observed no exception to this correlation though the degree varied from about 5 to 25 per cent increase.

This seems to indicate that the hen, like the mammalia, is equipped with some mechanism for mobilizing red cells in an emergency.

Consideration of the data presented in the right hand columns of table 1 shows that the splenectomy operation described completely inhibits this mechanism, while the control operation leaves it unaffected (see table 2). By analogy with work on mammalia we may presume that the inhibition is due to the removal of the spleen with its ability to expel its store of red cells into the general circulation.

We may conclude on the basis of this evidence that the reservoir function of the spleen is present in fowls as in mammals.

THE INDEPENDENCE OF SPONTANEOUS GASTRO-INTESTINAL MOTILITY AND BLOOD SUGAR LEVELS

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Considerable practical and theoretical interest is associated with the causal factors of spontaneous gastro-intestinal motility. Attempts have frequently been made to determine these factors, especially those concerned with the motility (hunger contractions) of the empty stomach. The evidence presented by Carlson (1919) indicating that gastric hunger motility may bear an inverse relation to the blood sugar level has attracted much attention.

Contradictory to this theory was the report of Templeton and Quigley (1930) that insulin hypoglycemia did not augment and intravenous injection of glucose did not inhibit spontaneous motility in the Heidenhain pouch or the pyloric pouch. A similar lack of relationship between blood sugar level and motility of the empty stomach was obtained with vitamin-B deficient dogs by Stucky, Rose and Cowgill (1928). Quigley and Lindquist (1930) found the hypoglycemia produced by phloridzin administration to be associated with a decrease instead of the anticipated increase in hunger contractions.

It was considered that under these experimental conditions the gastric motor mechanism was abnormal for Bulatao and Carlson (1924) had reported that intravenous administration of glucose to the normal dog was followed by a marked gastric inhibition, while insulin hypoglycemia was associated with gastric motor augmentation.

We have reinvestigated in detail the effect on gastro-intestinal motility of carbohydrate administration; gastric motility has received our chief emphasis, but the motility of the small intestine and colon has also been studied. The investigation included double vagotomized dogs as well as normal animals.

Twelve healthy dogs were selected for this investigation, five animals had experienced no surgical intervention, one had a colostomy, one had a cannula to the duodenum, two had a fistula into the terminal portion of the ileum and three had a double vagotomy and also a colostomy. Since our studies on gastric motility concerned factors which might modify this

motility, we usually carried out our experimental procedures when the motility was vigorous and was as continuous and uniform as possible.

Gastric inhibition can readily be produced by the manipulations incident to an intravenous injection. We endeavored by extensive training and careful routine manipulations of the animals to avoid the production of psychic disturbance throughout the entire experiment. We further emphasized physiological conditions by employing moderate amounts of physiologically isotonic solutions of the carbohydrates but we also employed larger quantities and also hypertonic solutions. In certain experiments we minimized the effect of pricking the skin by smearing the area with phenol paste. On other occasions we inserted into a leg vein a needle connected to a reservoir of 0.9 per cent saline. By gravity feed, saline was slowly passed through the needle to avoid an obstruction with clotted blood. At a suitable time the turning of a valve introduced glucose solution instead of saline into the vein. A period of about 20 minutes was utilized for the injection of 100 cc. We found it advantageous for the experimentor to leave the room at the beginning of this interval and watch the proceedings through a window. In the major portion of our experiments less elaborate precautions than those just described were employed, yet the results lead to similar conclusions. In all cases our solutions were at body temperature when administered. In each investigation the study was made repeatedly, usually under a variety of conditions so the results described as typical are believed to be uncomplicated by incidental factors. Gastro-intestinal motility was followed by the balloon method. "True" blood sugar determinations were made by the method of Somogyi (1929). Fasting periods varied from 18 to 64 hours at the beginning of the experiments.

Intravenous administration of glucose. In normal animals we determined on 71 occasions the effect on gastric motility of intravenous injections of glucose in quantities ranging from 1 to 25 grams. The conclusion appeared to be quite definite that hyperglycemia produced by these procedures was without immediate effect on spontaneous gastric motility. Three or four balloons in tandem, introduced into various parts of the stomach gave results similar to the single balloon and indicated that all parts of the stomach behaved similarly to intravenous glucose (fig. 1).

Occasionally a transient gastric inhibition was obtained just before, during, or after the injection. This inhibition was definitely associated with restlessness on the part of the animal,—it persisted for only 1 or 2 minutes (the original degree of motility returning while the hyperglycemia was very marked). An augmentation of motility associated with the injection was of approximately as frequent occurrence as this transient inhibition. Such augmentation or inhibition appears to be entirely incidental (related to psychic disturbance of the animal).

Another problem arising from this investigation concerns the late effects to be obtained from the administration of glucose. It has frequently been shown (c.f., e.g. Quigley, Hallaran and Barnes, 1932) that a period of

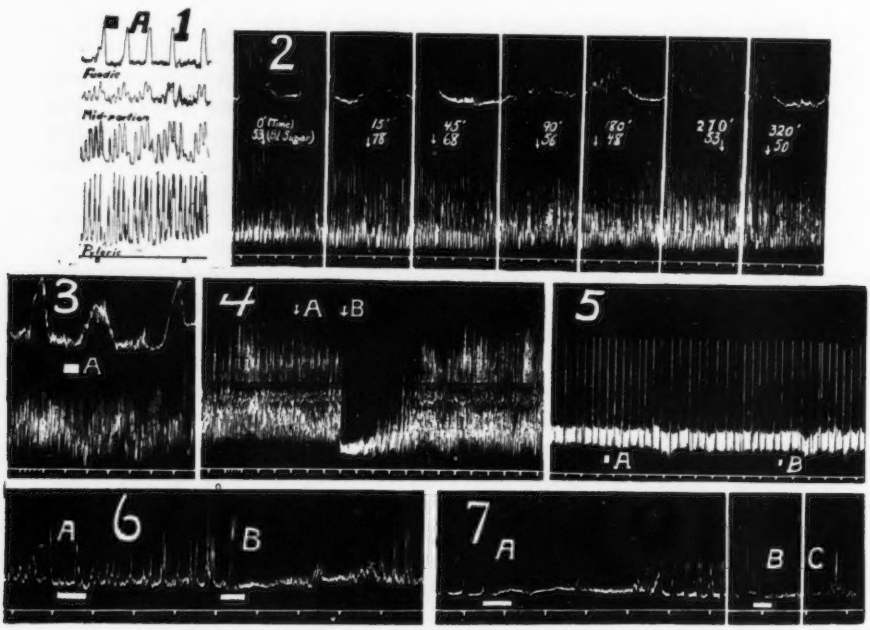

Fig. 1. Normal dog, 40 hours since fed, 4 balloons in stomach. A. Intravenous injection 10 grams glucose (30 cc.).

Fig. 2. Normal dog, 40 H.S.F., record from colon and stomach. At *O'* injected intravenous 0.25 gm/kgm. glucose (50 per cent).

Fig. 3. Normal dog, 34 H.S.F., record from ileum and stomach. At *A* injected intravenously 15 grams glucose (50 per cent). "True" blood sugar 10 minutes before injection; 79 mgm. per 100 cc., 60 minutes after injection 160.

Fig. 4. Normal dog, 18 H.S.F., record from stomach. A. Intravenous injection of 10 grams glucose (50 per cent). B. Intravenous injection of 0.5 cc. oxytocin.

Fig. 5. Vagotomized dog, 40 H.S.F. record from stomach. A. Intravenous injection of 50 grams glucose (50 per cent). B. Intravenous injection of 30 cc. of 0.9 per cent NaCl.

Fig. 6. Normal dog (with cannula to duodenum), 20 H.S.F., record from stomach. A. Fifty cubic centimeters 0.9 per cent NaCl into duodenum. B. Fifty cubic centimeters cane sugar (isotonic) into duodenum.

Fig. 7. Normal dog (with cannula to duodenum), 40 H.S.F., record from stomach. A. Fifty cubic centimeters glucose (5 per cent) into duodenum. B. Thirty-five minutes later, 50 cc. lactose (isotonic) into duodenum. C. Twenty-eight minutes later.

Time intervals 5 minutes.

hyperglycemia such as may be produced by administration of glucose is followed by an interval of hypoglycemia—supposedly the result of overproduction of insulin by the pancreas. It is well established that the administration of insulin to fasting animals will increase gastro-intestinal motility. The insulin produced physiologically in response to glucose administration might be expected to have a similar effect. Such a concept might explain why an individual experiences hunger 4 to 6 hours after a meal and the idea has already been employed by clinicians in prescribing the so-called "sugar breakfast" to augment hunger. Another method of attacking our problem was thus suggested since it is a procedure which modifies the blood sugar level.

We attempted to answer the questions here raised by observing the degree of gastro-intestinal motility during a period of physiological hypoglycemia, i.e., 3 to 5 hours following the administration of glucose. Results so obtained are admittedly difficult to evaluate for many extraneous factors may alter the degree of gastro-intestinal motility during an observation period of 5 hours. We were unable to make our observations more conclusive when we shortened the observation period. As the result of 63 experiments records were obtained in which an increase in gastric motility occurred during the period of hypoglycemia when the blood sugar values had fallen below normal and motility decreased as the blood sugar returned to the original level. Many records showed no such change (fig. 2) or even the reverse condition and our results only permit the conclusion that if the intravenous administration of glucose leads to a delayed increase in gastric motility the effect is slight and inconstant in its appearance.

Quigley and Solomon (1930) have shown that when hypermotility of the colon and small intestine occur during insulin hypoglycemia, complete inhibition of these portions of the gut follow the administration of glucose. We have investigated the effect of injections of glucose on *spontaneous* motility of the intestine and as the result of 17 experiments have concluded that intravenously administered glucose does not modify the *spontaneous* motility of the ileum or proximal or distal colon (figs. 2 and 3).

From the results obtained in 33 experiments we concluded that the intravenous injection of glucose produced neither inhibition nor augmentation of the spontaneous motility of the stomach or proximal or terminal colon in *double vagotomized* dogs, either immediately after the injection or later during the hypoglycemic period (fig. 5).

Administration of carbohydrate into the gastro-intestinal tract. The observation has repeatedly been made that the oral administration of food, especially carbohydrates, will readily relieve hunger and inhibit hunger contractions. Recognizing the possibility that this inhibition might be related to the production of hyperglycemia we have investigated the mechanism of gastric inhibition by carbohydrates introduced into the gut.

In an extended series of experiments we have found that the introduction of 0.9 per cent saline into the stomach in amounts even in excess of 100 cc. did not modify gastric motility in normal dogs. In 32 experiments introduction of glucose as an isotonic or hypertonic solution readily produced complete gastric inhibition which usually persisted for 13 to 50 minutes (fig. 8). This inhibition became evident within three minutes of

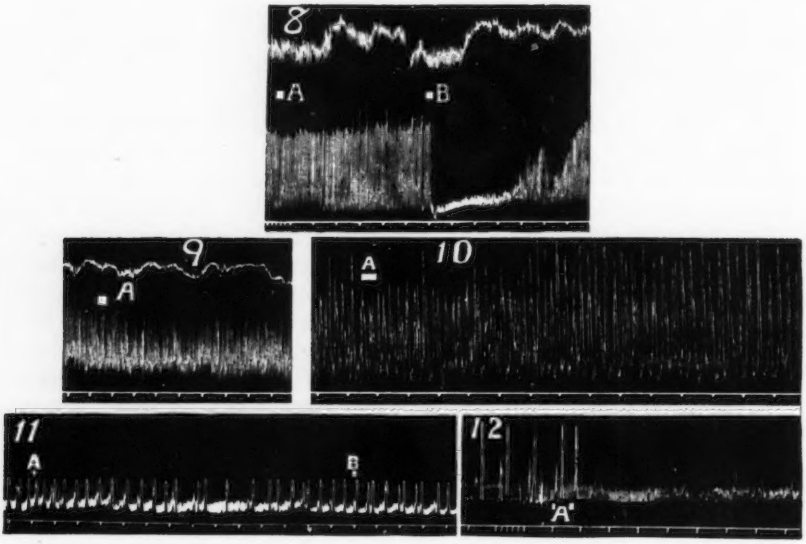

Fig. 8. Normal dog, 40 H.S.F., record from ileum and stomach. A. One hundred cubic centimeters 0.9 per cent NaCl into stomach. B. One hundred cubic centimeters 5 per cent glucose into stomach.

Fig. 9. Normal dog, 20 H.S.F., record from ileum and stomach. A. Fifteen grams glucose (50 per cent) into ileum.

Fig. 10. Normal dog, 40 H.S.F., record from stomach. A. Fifty cubic centimeters glucose (10 per cent) into colon, 20 cm. from external sphincter.

Fig. 11. Vagotomized dog, 40 H.S.F., record from stomach. A. One hundred cubic centimeters lactose (isotonic) into stomach. B. One hundred cubic centimeters 0.9 per cent NaCl into stomach.

Fig. 12. Vagotomized dog, 40 H.S.F. record from stomach. A. One hundred cubic centimeters lactose (isotonic) into stomach.

Time intervals 5 minutes.

the time administration began and was replaced by active motility at a time when glucose absorption and the attendant hyperglycemia would be reaching a maximum. It thus appeared very unlikely that the inhibition was related to the production of hyperglycemia.

Introduction of isotonic solutions of cane sugar (6 experiments) or lactose (30 experiments) into the stomach was also quickly followed by gastric inhibition. Since the hydrolysis and absorption of these disaccharides would require an appreciable interval, further evidence was provided in favor of the conclusion that gastric inhibition from carbohydrates introduced into the stomach was not the result of hyperglycemia. Typical gastric inhibition could be obtained by the introduction of carbohydrate solution into the stomach of a dog on whom the absence of effect from intravenous glucose had been demonstrated a few minutes earlier. Introduction of carbohydrate solution into the stomach did not modify motility of the terminal ileum or the colon (fig. 8).

Evidence was obtained by Quigley, Johnson and Solomon (1929) indicating that insulin gastric hypermotility was readily inhibited by glucose in the duodenum but not by glucose remaining in the stomach. In the present investigation it was possible that gastric inhibition following the introduction of carbohydrate into the stomach was related to carbohydrate in the duodenum but not to carbohydrate in the stomach. Evidence supporting this theory was obtained by passing a gold-plated cannula through the abdominal wall of a dog and attaching it to the peritoneal surface of the lower third of the duodenum. Solutions were introduced into the lumen of the duodenum by passing a long hypodermic needle along the cannula and through the duodenal wall. Introduction of physiologically isotonic glucose, lactose or cane sugar produced gastric inhibition entirely comparable to that following similar injections into the stomach (figs. 6, 7).

In vagotomized dogs the introduction of glucose (11 experiments), cane sugar (10 experiments) or lactose (8 experiments) into the stomach usually produced gastric inhibition similar to that observed in normal animals (fig. 12). Occasionally, however, gastric inhibition did not immediately follow such administration of carbohydrate to vagotomized animals (fig. 11). Retaining the theory that gastric inhibition is related to carbohydrate in the duodenum, we explained this variation in vagotomized animals as resulting from gastric retention of the carbohydrate due to the pyloric hypertonicity frequently observed in these animals.

It would appear that gastric inhibition following the introduction of carbohydrate in the upper gut is related to a reflex from the duodenum. This reflex probably is not mediated over vagus fibers since it can occur in vagotomized animals. This inhibition is probably similar to that demonstrated in the Heidenhain pouch (by Templeton and Quigley, 1930) following the introduction of glucose into the main stomach.

Absorption of glucose is believed to occur readily from the ileum and another method of investigating the effect of hyperglycemia on gastric motility was thus suggested. In 10 experiments we uniformly failed to influence gastric hunger contractions in normal dogs by the introduction of

25 to 50 cc. of glucose as a 5 to 50 per cent solution into the lower portion of the ileum (fig. 9). We likewise failed to observe appreciable changes in the blood sugar level in these experiments. Similar amounts of glucose introduced into the colon of normal or vagotomized dogs was also without effect on gastric motility (fig. 10).

We have confirmed the observation of Carlson that intravenous injection of 17 per cent saline or 0.45 gm./kgm. acetone does not modify motility of the empty stomach.

Discussion. Gastric hunger contractions can be augmented by fasting, moderate exercise, hemorrhage, exposure to cold, administration of insulin, pancreatic diabetes (Carlson and collaborators), or hepatectomy (La Barre and Destrée, 1930). Can these diversified procedures be correlated to yield a theory which is in accord with the reported observations and perhaps explain the underlying factors of gastro-intestinal motility? Luckhardt and Carlson pointed out that under the above conditions augmented gastric motility was associated with *a*, a decrease in the blood sugar concentration; *b*, a lowering of the glycogen reserve; *c*, an inability of the tissues to burn carbohydrates; *d*, acidosis of varying degree. Admittedly one or more of these four factors are encountered in each case mentioned but none are present in all the procedures augmenting gastric hunger contractions. Fasting, exposure to cold, hepatectomy or insulin administration will lower the blood sugar level, but hyperglycemia occurs in experimental diabetes and also may result from hemorrhage (Mulinos, 1928) and moderate exercise (Nolte, 1929). (Blood sugar values were not reported by Carlson in his experiments with hemorrhage and exercise.) A consideration of the problem from the standpoint of modifications in carbohydrate metabolism apparently does not supply the answer. Fasting and diabetes decrease carbohydrate metabolism; exercise, exposure to cold, insulin or intravenous glucose would augment carbohydrate metabolism. The conditions which would lead to gastric hypermotility are practically all conditions which would lower glycogen reserve. An exception is the case of insulin administration. Hypermotility begins here at a time when glycogen formation is augmented. Occurrence of hypermotility from pancreatic diabetes and also following insulin administration is especially difficult to harmonize. In depancreatized animals carbohydrate metabolism is depressed, R.Q. is decreased, metabolism of fats and proteins augmented, glycogen formation and storage in the liver decreased, formation of ketone bodies and tendency to acidosis increased. In the insulinized animal practically the opposite set of conditions exists. Perhaps we must assume in the insulinized animal a localized carbohydrate starvation for the hunger mechanism at a time when carbohydrate combustion of the body as a whole is augmented. This question of localized carbohydrate starvation deserves further investigation.

The above discussion should be considered in relation to the previously

reported observations that gastric hunger motility is not inversely related to the blood sugar level in the Heidenhain pouch, the pyloric pouch, the phlorhidzinized animal or the vitamin-B deficient dog. Combining this with our present observations that motility in the normal or vagotomized stomach is not modified by intravenous glucose and probably not by the hyperglycemia resulting from carbohydrate introduced into the gut, we feel that a satisfactory statement of the relation of carbohydrate to the control of gastric motility is still wanting.

Intravenous injections of hypertonic glucose solutions will produce marked changes in phosphate and calcium metabolism and also in osmotic relations between the blood, tissue cells and cerebrospinal fluid. It appears significant that such changes can be produced without modifying motility of the gut.

Rose, Stucky, Mendel and Cowgill (1931) have investigated the gastric atony which occurs in dogs deprived of water. They suggest that anhydremia is the cause of gastric atony. Our experiments in which marked changes in the osmotic pressure of the blood were produced by the intravenous injection of hypertonic solutions of saline or glucose fail to support this theory.

In attempting to explain the inhibition of the gastro-intestinal tract which follows the intravenous injection of pituitrin, vasopressin or oxytocin to dogs, circumstantial evidence was presented by Quigley and Barnes (1930) tending to show that the *latter part* of the inhibition period might be dependent on an elevation of the blood sugar level. Although it theoretically is not impossible that this may still be a factor in the case of dogs with insulin hypoglycemia, it certainly is not so in the normal dog. In the normal animal, not only does intravenous glucose fail to produce inhibition, but the administration of pituitrin preparations shortly after the glucose is immediately followed by the typical pituitrin inhibition (fig. 4). A similar situation holds for adrenalin: blood sugar elevation was considered as a possible factor in the inhibition of gastric motility following adrenalin administration (Quigley, Johnston and Solomon, 1929). However, in this case further evidence minimizing the importance of the blood sugar level is available in the observation of Wilder and Schultz (1930) that the blood sugar level is not elevated at the time adrenalin inhibits insulin gastric hypermotility.

Spontaneous motility of the empty stomach displays a striking similarity to insulin hypermotility. This is true to such a degree that physiologically produced insulin might be considered a factor controlling spontaneous gastric motility. Since post-insulin motility is completely inhibited by intravenously injected glucose but spontaneous motility is not modified by such injections, insulin production cannot be the controlling factor in spontaneous gastric motility. Furthermore, this difference in behavior

towards glucose affords a ready method of distinguishing post-insulin hypermotility from spontaneous motility of the empty stomach. Why intravenous glucose completely inhibits gastric motility in the insulinized animal but is without effect on spontaneous gastric motility has not been satisfactorily explained. Psychic factors may play a rôle for animals usually become restless when the marked insulin hypoglycemia is relieved by intravenous glucose.

Quigley, Hallaran and Barnes (1932) have shown that blood sugar levels or carbohydrate metabolism is not strikingly different in normal and chronic vagotomized dogs. This may be taken as further evidence that the depressed gastric tone and motility observed in vagotomized animals cannot be related to abnormalities in carbohydrate metabolism.

SUMMARY

Investigating the theory that spontaneous motility of the empty stomach is inversely related to the blood sugar level, we have in general obtained experimental results not in agreement with this theory.

We failed to modify gastric motility in normal or vagotomized dogs by the intravenous injection of glucose. Such injections also were without effect on motility of the terminal ileum or colon. The spontaneous hypoglycemia occurring several hours after glucose administration was without constant effect on motility of the stomach, ileum or colon. Glucose, cane sugar or lactose introduced into the empty stomach produced gastric inhibition in the normal or vagotomized dog. This inhibition was apparently not related to the production of hyperglycemia but appeared to be a reflex from the duodenum. Carbohydrates introduced into the duodenum lead to a similar gastric inhibition.

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FURTHER STUDIES OF THE FOLLICULAR-CORPUS LUTEUM HORMONE RELATIONSHIP IN THE RABBIT[1]

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In a previous paper (Hisaw and Leonard, 1), it was pointed out that the production of a progestational endometrium in the uterus of a castrate rabbit required the combined action of the follicular and corpus luteum hormones in which the follicular hormone had to exert its influence first. This "one-two" relationship was previously demonstrated for the decidual reaction in the uterus of rats and guinea pigs by Weichert (2) and for the corpus luteum hormone "relaxin" by Hisaw (3), (4) and Fevold et al. (5). This synergistic mode of action of the two ovarian hormones to produce the uterine changes in the rabbits has been upheld by Allen (6), Clauberg (7), (8) and Parkes (9). It was also shown in the previous communication (1) that the corpus luteum hormone "corporin" (Hisaw) "progestin" (Corner) responsible for progestational changes could not maintain indefinitely the characteristic histological picture of early pregnancy in the uterus of castrate rabbits. Also it was demonstrated by injections of follicular hormone and corporin that it was possible to prevent the progestational modification of the uterus by sufficient quantities of the follicular hormone. In this paper we wish to present further data on the oestrin-corporin relationship and to discuss certain other physiological aspects which have developed from the experiments to be reported.

MATERIAL AND METHODS. The corpus luteum hormone responsible for the progestational reaction has been termed "corporin" in this laboratory and is the same active substance used to produce premenstrual changes of the endometrium in the uterus of castrate monkeys (Hisaw, Fevold and Meyer, 10). It is also essentially similar to Allen's (11) preparation "progestin."

Since the discovery of the separation of the corpus luteum hormones, (Fevold et al., 12) a choice of methods of preparation of corporin may be made depending on whether or not it is desired first to recover the relaxative hormone. These methods are given in full in a recent publication

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(Fevold et al., 13). The corporin which was used in the following experiments was extracted from the corpus luteum tissue by hot acid alcohol and purified as described (13). The amount of corporin necessary to produce a good progestational modification in the uterus of rabbits castrated in heat is equivalent to 25 to 50 grams of fresh tissue. This preparation was practically free of oestrin in that 25 to 50 grams equivalent were not sufficient to bring a castrated female rat into heat although 75 grams apparently contained sufficient amounts to do so. It is preserved in 100 per cent alcohol in stock solution, dissolved in corn oil as a vehicle when ready for injections, and only sufficient quantities are prepared in oil to last for five days. Several batches of corporin were employed in these experiments but in attempting to examine the relationships of oestrin and corporin in a partially quantitative way a single batch was used. The follicular hormone employed was oil soluble theelin and was carefully restandardized just previous to using.[2]

Sexually matured female rabbits ranging in weight from $2\frac{1}{2}$ to $3\frac{1}{2}$ kilograms were isolated for 15 days or more before they were used in these experiments. Thirty-five animals were employed in the tests. Injections of corporin were made twice daily, follicular hormone once daily, and the two hormones when used together were always injected separately. In describing the degree of uterine glandular development, it was decided to abide as closely as possible to the standard set down by Allen (11) which consists of four degrees, +1 to +4. A rabbit unit of the active principle is defined as that amount of extract which will produce in 5 days a uterine modification, in an adult rabbit castrated in heat, equal to that normally occurring on the 6th to 8th day of pregnancy (+3 to +4).

Experimental. It was pointed out in a previous paper (1) that corporin alone when given over an extended period, would not maintain the characteristic histological picture of early pregnancy as produced in 5 days. This experiment has been extended further to confirm our original suggestion. For example, corporin was injected in doses of 1 to 4 rabbit units for each 5 days for a total of 15 days, samples of the uterus being removed and sectioned every 5 days. The results clearly showed that no matter how active the extract, it was impossible to prolong the modification beyond 10 days. A collapse of the glands could be seen by the 10th day and retrogressive changes in the cells lining the lumen of the glands of the uterus could be demonstrated. By the 15th day, the uterine glands had almost completely disappeared, their lumens had become occluded, and numerous leukocytes had invaded the endometrium. The tall columnar epithelium so characteristic of the active glands became very low and the ends of the cells much frayed out. The nuclei of the epithelial layer

[2] The theelin was kindly furnished by Parke, Davis & Co.

instead of being elongated as in the first five days of treatment became rounded and pycnotic by the 15th.

TABLE 1

*Effect of varying doses of theelin and corporin on progestational modifications of the uterine endometrium of rabbits**

RABBIT	TREATMENT (5 DAY PERIODS)	RESULT OR DEGREE OF UTERINE MODIFICATION
RA130	1. 50 grams tissue equivalent	1. +3
RA134**	1. 75 grams tissue equivalent 2. Injections continued 5 more days at same level	1. +4 2. Glands regressing
RA131	1. 1 Rb.U. corporin plus 50 R.U. theelin	1. In heat
RA132	1. 1 Rb.U. corporin plus 25 R.U. theelin	1. In heat
RA143	1. 1 Rb.U. corporin plus 25 R.U. theelin 2. Injections continued 5 more days at same level	1. +1 2. Few glands, endometrium in heat
RA147	1. 1 Rb.U. corporin plus 25 R.U. theelin 2. Injections continued 4 days, theelin stopped, corporin injected 5 days	1. In heat 2. +2 to +3
RA144	1. 1 Rb.U. corporin plus 10 R.U. theelin 2. Injections continued 5 more days same level	1. In heat 2. In heat
RA148	1. 1 Rb.U. corporin plus 10 R.U. theelin	1. In heat
RA150	1. $1\frac{1}{3}$ Rb.U. corporin plus 10 R.U. theelin 2. $2\frac{2}{3}$ Rb.U. corporin plus 10 R.U. theelin	1. +2 to +3 2. Glands regressing
RA146	1. $3\frac{1}{3}$ Rb.U. corporin plus 25 R.U. theelin	1. +2
RA149	1. 2 Rb.U. corporin plus 25 R.U. theelin 2. Corporin same 5 more days; theelin 5 R.U.	1. +2 to +3 2. Glands regressing
RA153	1. $5\frac{1}{3}$ Rb.U. corporin plus 25 R.U. theelin	1. +2 to +3

* Rabbits castrated in heat, injections begun immediately.

** 75 grams = 1 Rb.U. All experiments in table 1 from same extract.

Knaus (14) while studying the changes in the sterile horn of unilaterally pregnant rabbits found that the endometrium also went through similar degenerative stages but by the time of parturition, had returned to the

oestrous condition. Courrier and Kehl (15) and Courrier (16) have shown that fresh corpora lutea produced with anterior pituitary material in the unilaterally pregnant rabbit could not induce new glandular formation in the sterile horn.

Empirically, the fact that oestrin could over-ride the corpus luteum hormone when both were injected simultaneously into castrate rabbits was demonstrated previously by Hisaw and Leonard (1). In the experiments reported here an attempt is made to establish a quantitative balance between the two and to demonstrate their action when competing for physiological control of the endometrium. The results given in table 1 are from the same stock solution so that this series of experiments was performed without changing extracts. It was found that 50 grams equivalent of this extract would produce a +3 reaction in the uterus of a rabbit castrated in heat (RA130), but in order to guard against any

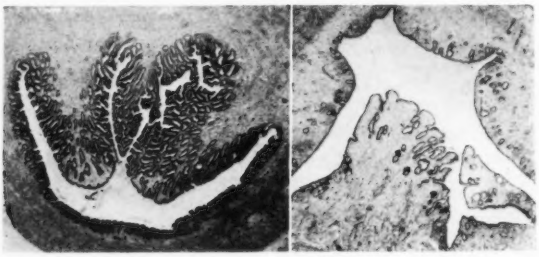

Fig. 1 Fig. 2

Fig. 1. RA150–1. Rabbit castrated in heat, received 1½ rabbit units of corporin plus 10 R. U. of theelin. Corpus luteum effects indicated.

Fig. 2. RA144–1. Rabbit castrated in heat, received 1 rabbit unit of corporin (75 grams tissue equivalent) plus 10 R. U. of theelin. Oestrin effects indicated.

possible variations, an equivalent of 75 grams of tissue giving a +4 reaction (RA134) was taken as the rabbit unit. Several preliminary trials were made before the final series of experiments in order to approximate the amount of theelin necessary to prevent the action of a given amount of corporin.

One rabbit unit of corporin (75 grams equivalent) was injected with 50, 25 and 10 R.U. of theelin for 5 days into rabbits castrated in heat. Ten rat units of theelin seemed to be the smallest amount to prevent the action of one rabbit unit of corporin, for in one of the animals receiving this amount (RA148) the cells of the uterine glands had only just begun to undergo the characteristic progestational change (fig. 1). But when a third of a rabbit unit more of corporin was injected with the 10 R.U. of theelin, a definite and unmistakable progestational reaction resulted

(RA150) (fig. 2). The injections were continued for 5 more days and the dosage of corporin was doubled, yet in spite of this, the usual degenerate changes in the uterine glands occurred.

When the dosage of corporin was raised to $3\frac{1}{3}$ rabbit units and the theelin to 25 R.U. the effect of corporin unmistakably superseded that of the theelin to give a +2 reaction (RA146). Similarly, two rabbit units of corporin and 25 R.U. of theelin gave a positive progestational modification (RA149). By lowering the dosage of theelin to 5 R.U. and keeping the amount of corporin constant for 5 more days, it was found impossible to prevent the onset of the degenerative changes on the 10th day. In the last case (RA153) a massive dose of $5\frac{1}{3}$ rabbit units of corporin was given along with 25 R.U. of theelin which resulted in a +2 to +3 modification. It was observed that whenever the combined injections were made and the corporin was more powerful in its influence, the end result was never better than a possible +3 reaction. Upon histological examination of the uterus of animals injected with both preparations simultaneously, a greater engorgement of the lymphatics and capillaries could be observed. This was indicated particularly when the theelin was more effective than corporin in the test animal.

An interesting result was obtained in rabbit RA147 which received one rabbit unit of corporin plus 25 R.U. of theelin for 5 days and then had a piece of the uterus removed and sectioned. The histological examination revealed an endometrium of an animal in heat with very much enlarged capillaries and lymphatic channels. The dosage of oestrin and corporin was kept constant for 4 more days, then the oestrin injections were stopped and the corporin continued for 5 more days at the same level. The result was a progestational endometrium of +2 to +3. Evidently, the oestrous hormone kept the uterus tuned up, so to speak, in the presence of sufficient corporin which alone could have produced a typical reaction in the first five days. Such experiments will explain much of the so-called antagonism of the ovarian hormones.

Further, it has been demonstrated by a number of workers that oestrin will terminate the early stages of pregnancy quite easily. Courrier (15) has shown that while small amounts of oestrin will terminate the early stages of pregnancy in rabbits, it takes large amounts (500 to 700 R.U.) to prevent the normal progestational changes which appear in the endometrium in the first 5 to 8 days. To test this, four rabbits were bred and given 50, 100, 200, and 500 R.U. of theelin respectively over a period of five days and pieces of the uteri were removed and sectioned. The results confirmed those of Courrier. No effects on the endometrium of the rabbits receiving 50 and 100 R.U. respectively could be determined, the one receiving 200 R.U. gave a +1 to +2 reaction. However, the uterus of the rabbit injected with 500 R.U. of theelin was completely negative,

the corpora lutea being unable to produce the modification in the presence of such large amounts of the oestrus-producing hormone. It is also difficult to inhibit progestational development with theelin during pseudo-pregnancy as shown by the following experiment. A rabbit which was in heat received 300 R.U. of theelin in three days, was allowed to copulate and then was given 125 R.U. during the next 5 days. A lutein reaction in the endometrium of +2 to +3 resulted in spite of the preliminary treatments of large amounts of theelin. These results show (1) that large doses of theelin may not inhibit ovulation, and (2) that there is a significant difference between the amounts of theelin needed to blot out the normal progestational picture as compared to the amount it takes to over-ride a rabbit unit of injected corporin.

Since it was possible to inhibit the normal progestational modification with large amounts of theelin, the question arose as to whether or not the

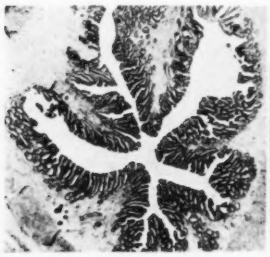

Fig. 3

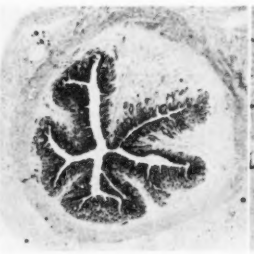

Fig. 4

Fig. 5

Fig. 3. RA88–1. Rabbit castrated in heat, injected with corporin for 5 days. Progestational changes present.

Fig. 4. RA88–2. Same animal on tenth day, the injections of corporin continued at same level. Note involution of glands.

Fig. 5. RA88–3. Same animal on fifteenth day; the injections of corporin continued at same level. Marked involution of glands.

concentration of oestrin was sufficiently high in the normal rabbit in heat to readily over-ride any injected corporin. If the rabbit has been isolated for 15 to 20 days, it is possible to elicit an apparently normal development of the uterine glands in 5 days with corporin. For example, RA138 was given 2 rabbit units of corporin for 10 days and a section of the uterus removed on the 5th and 10th day. The endometrium was diagnosed as +4 on the 5th day and degenerative changes, though not marked, had begun by the 10th day. But this reaction cannot be considered as positive if by handling the animals or in some manner ovulation occurs because, in such cases, the animals' own corpora lutea would defeat the purpose of the test. However, the ovaries of all animals so injected contained small

follicles and never any recently formed corpora lutea. It was also interesting to note that ovulation could be induced in rabbits on the 5th day of corpus luteum treatment by the injection of the urine of pregnant women after the manner described by Friedman (17).

Klein (18) also has been able to induce progestational modifications in the endometrium of non-castrate female rabbits but claims that it is more difficult to do so. To test this, a rabbit unit of corporin which would produce a +3 reaction in the uterus of a rabbit castrated in heat was injected into a non-castrated animal with the result of only a +1 reaction. However, when the dose was raised to 1½ rabbit units a +4 reaction resulted. This experiment is very suggestive of the nature of the follicular hormone content of the rabbit in heat. A further discussion of these several problems is given below.

Discussion. From the first series of experiments in which rabbits castrated in heat received corporin for 15 days, it is seen that regression of the endometrial glands occurs in spite of ample amounts of the hormone (figs. 3, 4, 5). This is not at all surprising if one considers the follicular hormone as a growth promoter and sensitizer of the uterus and corporin as a modifier of structures already formed by the follicular hormone. The effects of the follicular hormone alone in producing the hyperemic condition of the uterus and growth of both the muscle and the endometrium are well known. When the source of this hormone is removed from the animal as after castration, retrogressive changes toward the castrate condition follow, but if the lutein hormone is present, it can modify the uterus during the process. This is probably what occurs normally during the early pregnant or pseudopregnant period of the rabbit's cycle in which the follicular hormone is probably at a very low ebb shortly following ovulation, and the lutein hormone exerts its influence without further interference from the oestrus-producing hormone. It is suggested that progestational "modification" be substituted for "proliferation" used heretofore as the latter does not signify the nature of action of the luteal hormone.

Knaus (14) claims that the uterine glands of the pregnant rabbit secrete longer than the pseudopregnant animal as the result of the larger size of the corpora lutea of pregnancy and that the retrogressive processes of the glands in the pregnant animal commence about the 15th to the 17th day of gestation. In the castrate animal injected with corporin, the atrophic changes are first seen on about the 10th day of injections but by the 15th day the glands have for the most part disappeared. It is the opinion that the difference in appearance of the degenerative changes in the experimental and pregnant animals is more apparent than real because of the conditions of the experiment. It must be remembered that in the experimental animal, from the time of castration in heat, corporin is available

in very high concentration while in the pregnant animal some time elapses before the corpora lutea reach a sufficient size to be fully active. This may account for the apparent earlier involutionary process of the glands in the injected animal. In the pseudopregnant rabbit, other factors as well may enter in to cause the earlier degenerative changes to appear, i.e., the onset of the next heat period.

Courrier (15) has demonstrated that the production of fresh corpora lutea in the ovaries of a unilaterally pregnant rabbit would not induce anew the endometrial changes characteristic of the first stages of pregnancy. This seems to be of great significance since Allen and Corner (19) have demonstrated that progestin will maintain pregnancy to term in a rabbit castrated 18 hours after copulation. The same extract that will produce the early changes of pregnancy is necessary for the maintenance of pregnancy and the life of the placenta. These facts make one speculate as to other possible factors in the lutein hormone necessary for the maintenance of pregnancy or the relation of the placenta to this hormone.

Further evidence that oestrin in the doses given would not prolong the progestational picture in the endometrium is observed from the experiments on simultaneous injections of corporin and theelin. The degenerative changes can be seen on the 10th day in spite of the presence of both hormones. More interesting still, is the fact that the result to be expected from simultaneous injections of both hormones depends on the amount of either given. In other words, a physiological antagonism exists between the two. The word "antagonism' has been used rather loosely in speaking of hormone interrelations but now it is possible, at least in this case, to further explain the nature of this opposing action.

If the amount of follicular hormone is physiologically greater than corporin, the endometrium remains in the oestrous state; if the corporin is physiologically greater in amount than the follicular hormone, the glands undergo their modification as they would normally. The two hormones then react towards one another as two different substances competing for the cells of the endometrium, either to maintain them as they are (pre-secretory phase) or modify them into the secreting phase characteristic of early pregnancy. A good example to illustrate the nature of the interaction of corporin and theelin is to be found in rabbit RA147 which received simultaneous injections of both hormones. The dosage was so given that at the end of the first five days, the theelin was stronger than the corporin and the oestrous condition was maintained in the uterus. This treatment was continued for 4 more days making a total of 9 and then the theelin was discontinued and the corporin administered at the same level for 5 more days. The result was that with the discontinuance of theelin, the corporin could still produce its modifications on the endometrium.

The literature contains many conflicting statements regarding antagonism of the ovarian hormones. Courrier (20) has shown the antagonistic reaction of oestrin on the corpora lutea of the guinea pig using the production of deciduomata as a test point. Angelvitz and Sterling (21) have stated there is no antagonism between progestin and oestrin using the vaginal mucification of the rat as a test. Patel (22) studying the same reaction has a very suggestive explanation of the relation of a lutein hormone and oestrin in that the lutein hormone reduces the activity of the vagina to oestrin and that it is the oestrous hormone which produces mucification. Parkes (9) claims that oestrin is not antagonistic to the uterine changes and that it aids in the progestational reaction. This is perhaps true in part but nevertheless the effects of corporin can be clearly destroyed provided sufficient oestrin is present.

The work of Courrier (16) and others in injecting large doses of oestrin to over-ride the action of normal corpora lutea has been confirmed. It is interesting to compare the amounts of oestrin that prevent the progestational development in the normal animal (500 R.U.) and the amount needed to override a rabbit unit of corporin (10 R.U.). The reason for this cannot be explained at present but one suggestion is offered in that the animal's own corpora lutea may produce many times the amount of corporin needed to affect a progestational reaction.

It has been demonstrated also that progestational changes can be produced with corporin in an adult non-castrate rabbit in heat. Klein (18) claims that it is more difficult to produce the reaction in such animals but does not say how much more hormone is needed. In the experiments reported, it was apparently necessary to use only a half of a rabbit unit more of corporin to produce a positive reaction. Klein also states that prolonged injections caused follicular atresia but in these experiments, the amount of corporin needed to produce a uterine modification in 5 days did not interfere with the ability to induce ovulation following the proper stimulation.

The experiment of inducing progestational modifications in the adult non-castrate rabbit permits one to speculate as to the concentration of the follicular hormone in the rabbit during heat. Assuming that it takes only a third of a rabbit unit more of corporin to override 10 R.U. of theelin in a castrate animal and only a half of a rabbit unit more to produce the reaction in the non-castrate female, one could conjecture that the concentration of the follicular hormone was a little better than 10 R.U. in the rabbit in heat. This is probably by no means accurate but at the same time does not seem entirely fantastic.

Our own studies and those of others indicate that the endometrium of the rabbit uterus cannot respond to both corporin and theelin at the same time but shows the effects of one or the other depending upon which

hormone has a quantitative advantage. The point at which the shift occurs is not sharp and in several respects resembles the results of a mass reaction. It also seems that the endometrium is conditioned by theelin and is depleted by secretion in response to corporin and that both processes cannot occur simultaneously in the same gland cell. We also have unpublished data which suggest that this explanation may also apply to the premenstrual endometrium of monkeys.

SUMMARY

1. It has been definitely shown that a quantitative relationship exists between oestrin and corporin in the production of endometrial changes characteristic of early pregnancy.

2. If oestrin and corporin are injected simultaneously into castrate rabbits, the result to be expected depends on the higher dosage of either hormone and the apparent antagonism is explained on the basis of the two hormones competing physiologically for the endometrium.

3. The oestrous hormone can hold the action of injected corporin in abeyance for an extended period (9 days at least) after which, if the dosage of oestrin is lowered and corporin treatment continued, the effect of corporin becomes manifested.

4. It takes many more times the amount of oestrin to obliterate the characteristic modification in the endometrium of early pregnancy than it does to overpower the effects of a rabbit unit of corporin capable of producing a histological picture equally as good in a castrate animal.

5. It takes slightly more corporin to produce a progestational modification in a non-castrate female rabbit in heat than it does to produce a comparable change in the uterus of a rabbit castrated in heat and then treated.

6. Corporin alone or with the addition of oestrin does not seem able to maintain the progestational modification indefinitely.

7. The amount of corporin which in 5 days will produce a good progestational modification in a non-castrate rabbit in heat will not inhibit ovulation following the proper stimulation.

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THE TRANSFER OF BICARBONATE BETWEEN THE BLOOD AND TISSUES CAUSED BY ALTERATIONS OF THE CARBON DIOXIDE CONCENTRATION IN THE LUNGS

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Henderson and Haggard (1918a, b) showed by acute experiments on dogs that alterations in the carbon dioxide tension of the alveolar air induced by breathing air enriched by carbon dioxide, or by over-ventilation, were followed by alterations in the bicarbonate reserve of the blood. The duration of these experiments was measured by minutes and must not be confused with experiments in which the duration is measured by days. The changes referred to were made apparent by a change in the level of the carbon dioxide absorption curve of whole blood: when the carbon dioxide tension of the alveolar air was raised, the level of the carbon dioxide absorption curve was raised; and when the alveolar carbon dioxide tension was lowered, the level of the carbon dioxide absorption curve was lowered. In this manner the ratio $\left(\frac{H_2CO_3}{BHCO_3}\right)$ tended always to approximate the normal value, bicarbonate passing from the tissues into the blood when the dissolved carbon dioxide was abnormally high, and from the blood into the tissues when the dissolved carbon dioxide was abnormally low. This principle has been widely accepted as a compensatory device, the function of which is to maintain the hydrogen ion concentration of the blood at the normal level.

Collip and Backus (1920) examined the plasma from 15 human subjects taken after 20 to 40 minutes of forced breathing, accompanied by an average fall in the alveolar carbon dioxide tension from the normal of 46 mm. to 22.5 mm. They found that plasma taken before over-ventilation combined with 66.6 volumes per cent of carbon dioxide when equilibrated with alveolar air, and immediately following the period of over-ventilation combined with only 55.6 volumes per cent of carbon dioxide when equilibrated at the same carbon dioxide tension. The results of these experiments on man are in substantial agreement with those of Henderson and Haggard (1918b) on dogs.

Grant and Goldman (1920) found that when the alveolar carbon dioxide tension in man was lowered from 41 to 23 mm. of mercury by voluntary

forced breathing, the carbon dioxide combining capacity of the plasma fell from 59.5 to 44.9 volumes per cent.

Davies, Haldane and Kennaway (1920) found that when the alveolar carbon dioxide tension in man was increased by breathing 5.2 to 6.4 per cent carbon dioxide in air for a period of 2 hours, the level of the carbon dioxide absorption curve was unaltered; nor was any change produced by lowering the alveolar carbon dioxide tension to about 11 mm. by voluntary forced breathing for 1 hour.

In view of the assumed efficacy of this mechanism in maintaining the acid base balance of the body, we have undertaken a series of experiments on both dogs and cats, which we hoped would throw further light on the extent and causes of the changes observed. In all of our experiments we found that the changes were either non-existent or in the reverse direction of those reported by Henderson and Haggard.

We shall avoid using the term "alkali reserve" in this paper, since the alkali in the blood is always in use and never in reserve. For our purposes the term "bicarbonate level" is more explicit, because any change in the alkali content of the blood will be made apparent by a shift in the level of the carbon dioxide absorption curve.

METHOD. The animals used were cats and dogs. A 10 per cent solution of barbital sodium was allowed to run slowly from a burette into the femoral vein, which had been exposed under ether anesthesia. The dose varied from 0.3 to 0.4 gram per kilo of body weight. A cannula was inserted in the trachea to facilitate the withdrawal of alveolar air samples, and another in the femoral or carotid artery for the withdrawal of blood samples. The animal was then permitted to rest for at least 3 hours before the first sample of blood was taken. This interval of time was considered necessary in order that a condition of stable equilibrium might be attained following the administration of the anesthetic and the operative procedure.

The alveolar carbon dioxide tension was raised by the inhalation of a gas mixture containing about 10 to 11 per cent carbon dioxide, 25 per cent oxygen, and nitrogen. The gas mixture was allowed to flow into a spirometer, from which the gas was withdrawn by the animal through respiratory valves.

The alveolar carbon dioxide tension was lowered by over-ventilation, by using either a positive pressure pump which was connected to the tracheal cannula or by using a respirator which induces the normal movements of the chest, similar to that described by Shaw and Drinker (1929) for the administration of artificial respiration to children. The rate of the apparatus was adjusted to fill the lungs about 25 times per minute.

Alveolar air was withdrawn for carbon dioxide determination by the method described by Shaw and Messer (1930). Samples were taken while

the animal was breathing normally and again during the period of altered alveolar carbon dioxide tension.

The blood samples were oxalated in air and then immediately introduced into tonometers containing about 25 per cent oxygen and carbon dioxide in such concentration that, after the equilibration had been completed, a sample of gas from the tonometer had a carbon dioxide tension of 40 ± 3 mm. Hg. The carbon dioxide content of the blood, as determined by the method of Van Slyke and Neill (1924), was then corrected to that which would exist if the carbon dioxide tension were exactly 40 mm. The correction thus applied, which was based upon the average slope of the carbon

TABLE 1

Control determinations of the carbon dioxide content of blood following equilibration at a given tension of carbon dioxide

EXPERIMENT 28			EXPERIMENT 30			EXPERIMENT 49		
Weight 9.3 kgm.			Weight 11.2 kgm.			Weight 7.4 kgm.		
Minutes	CO_2 at 40 mm.	Averages	Minutes	CO_2 at 40 mm.	Averages	Minutes	CO_2 at 40 mm.	Averages
Air			Air			Air		
	vols. per cent			*vols. per cent*			*vols. per cent*	
			5	43.2				
30	49.2		35	43.1		30	41.7	
60	49.3		65	43.2		60	42.9	
82	48.5		95	42.5		90	43.2	
111	49.4		125	42.6		120	42.7	
140	49.0	49.1	155	42.8	42.9	150	42.3	42.5

TABLE 2

Control determinations of the constancy of the carbon dioxide combining capacity of the blood of dogs which are resting under experimental conditions

EXPERIMENT 22			EXPERIMENT 27			EXPERIMENT 29		
Weight 8.4 kgm.			Weight 15.2 kgm.			Weight 12.7 kgm.		
Minutes	CO_2 at 40 mm.	Averages	Minutes	CO_2 at 40 mm.	Averages	Minutes	CO_2 at 40 mm.	Averages
Air			Air			Air		
	vols. per cent			*vols. per cent*			*vols. per cent*	
0	43.4		0	39.4		0	43.2	
30	39.6		20	41.2		30	42.5	
60	43.8		40	42.1		60	43.8	
90	43.5		60	42.0		90	43.0	
120	43.4		80	42.1		120	43.3	
150	42.8	42.8	100	41.9	41.4	150	43.7	43.2

dioxide absorption curve of the animal's blood, was subject to an error so slight as to be negligible.

Since the determination of the carbon dioxide content of blood following equilibration at a given tension of carbon dioxide involves several procedures, each of which is subject to error, we have shown the results of three control experiments in table 1 so that the effective error may be correctly appraised. About 60 cc. of blood were withdrawn from a dog and 10 cc. introduced into each of 5 or 6 tonometers. To test for the possible formation of lactic acid due to standing at room temperature, the samples were equilibrated and transferred to the mercury collecting tubes at intervals of 30 minutes. The last sample was, therefore, exposed to the effect of room

temperature for about 145 minutes longer than the first sample. No change in its carbon dioxide combining capacity was observed. The process of equilibration plus carbon dioxide analysis is subject to an error of ± 0.7 volume per cent, or a disagreement of 1.5 volumes per cent between the minimum and maximum values of a series. The effect of an error, however, is greatly minimized by the fact that the results of an experiment are given in terms of averages taken from two or more observations.

The constancy of the bicarbonate level in the blood under the conditions of our experiment is shown in table 2. In these control experiments dogs were anesthetised, prepared, and allowed to rest for 3 hours before the first

TABLE 3

The respiratory volume of dogs under experimental conditions

EXPERIMENT	WEIGHT	NATURAL ALVEOLAR CO_2	NATURAL RESPIRATORY RATE PER MINUTE	RESPIRATORY VOLUME (CC. PER MINUTE PER KILO BODY WEIGHT)		
				Natural	High CO_2	Over-ventilation
(1)	(2)	(3)	(4)	(5)	(6)	(7)
	kgm.	*mm.* Hg				
44	12.2	46	8	158		485
43	12.5	49	8	172	590	
37	18.9	45	14	200	980	
47	9.0	46	25	234		1,190
45	11.4	46	16	246		780
56	9.9	46	19	256		1,160
31	19.6	43	19	260	653	
35	18.0	40	25	272	1,280	
36	15.4	40	22	280	1,240	
34	13.5		22	288	845	
32	10.2	43	20	304	1,285	
46	12.2	35	29	360		1,230
48	8.5	34	31	360		1,300
33	17.0	38	60	512	1,310	

blood sample was taken, in the manner already described. Blood samples were then taken while the dog was breathing normally at intervals during a period of time equivalent to the duration of our regular experiments to determine the range of variation in the bicarbonate level which might be expected to occur quite irrespective of alterations in the alveolar carbon dioxide tension. While the prevailing level remains very steady, the extreme variation is greater than the analytical error, owing to an occasional fall in the bicarbonate level which is only transitory in effect.

It was found that the average rectal temperature taken directly after the administration of the anesthesia was 39.0°C. in the dog and 38.3°C. in the cat. These temperatures were held constant within ± 0.5°C. through-

out the experiment by the use of artificial heat. After a few minutes of hyperventilation, caused by the inhalation of carbon dioxide or by enforced over-ventilation, the body temperature started to fall, but was instantly checked by increasing the external heat.

The temperature of the bath in which the blood was equilibrated was held at 37°C. Since this is 1.3 to 2.0°C. lower than the body temperature of the animal from which the blood was taken, the carbon dioxide combining capacity of the blood as determined by us must be subjected to a slight correction. But, owing to the fact that all the data presented are concerned with the relative values of the carbon dioxide combining capacity and not with the absolute values, the correction becomes superfluous.

In table 3 the respiratory volume of the dogs is given in terms of cubic centimeters per minute per kilo of body weight. The data are arranged in order of the increasing values of the natural respiratory volume (column 5), which was determined just prior to the induced change in the alveolar carbon dioxide tension (columns 6 and 7). It will be observed that the alveolar carbon dioxide tension varies from 49 to 34 mm. of mercury. We do not know the alveolar carbon dioxide tensions of the normal intact dog, but it is probable that the fluctuations which appear under the conditions of our experiments are greater than the fluctuations which would occur in the intact dog. Immediately following the intravenous injection of barbital sodium the respiratory rhythm is usually temporarily disturbed. It may, however, even after 2 or 3 hours, remain abnormally rapid or abnormally slow, which in turn is responsible for the extremes which occur in the alveolar carbon dioxide tension.

The response to over-ventilation (column 7) was governed entirely by the respirator. The response to the carbon dioxide inhalation (column 6), on the other hand, depends upon the respiratory center. Low responses to the inhalation of carbon dioxide were observed in experiments 31, 34 and 43. It was noted that dog 31 was more profoundly anesthetised than the other animals, and that number 34 was recovering from distemper. These may have been contributing causes of the inadequate response to the carbon dioxide stimulus.

EXPERIMENTAL RESULTS. *The effect of raising the alveolar carbon dioxide tension upon the bicarbonate level of blood.* The effect upon dogs' blood is given in table 4. The manner of tabulating our data can best be shown by reviewing in detail experiment 32 in table 4. The time in minutes following the first blood sample is given in column 1. Two blood samples were taken with 30 minutes intervening while the dog was breathing room air. Thirty-one minutes after the first sample the respiratory valves were connected to a spirometer containing the carbon dioxide gas mixture. The alveolar carbon dioxide tension was raised from 43 mm. to 83 mm., and the average bicarbonate level of the blood fell from 46.2 volumes per cent

TABLE 4

The transfer of bicarbonate between the blood and tissues of dogs, caused by increasing the carbon dioxide concentration in the lungs

EXPERIMENT 32				EXPERIMENT 33				EXPERIMENT 34				EXPERIMENT 35				EXPERIMENT 36				EXPERIMENT 37				EXPERIMENT 43				EXPERIMENT 31			
Weight 10.2 kgm.				Weight 17 kgm.				Weight 13.5 kgm.				Weight 18 kgm.				Weight 15.4 kgm.				Weight 18.9 kgm.				Weight 12.5 kgm.				Weight 19.6 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air				Air				Air				Air				Air				Air				Air				Air		
	vols. per cent		*mm. Hg*		*vols. per cent*		*mm. Hg*		*vols. per cent*		*mm. Hg*		*vols. per cent*		*mm. Hg*		*vols. per cent*		*mm. Hg*		*vols. per cent*		*mm. Hg*		*vols. per cent*		*mm. Hg*		*vols. per cent*		*mm. Hg*
0	45.7			0	43.5			0	44.6			0	46.3			0	43.5			0	41.6			0	Lost			0	40.4		
30	46.8	46.2	43.0	30	44.9	44.2	38.0	30	43.7	44.1		30	45.4	45.8	40.0	30	43.2	43.3	40.0	30	42.9	42.2	45.0	30	46.9	46.9	49.0	30	40.2	40.3	43.0
31	High CO_2			35	High CO_2			35	High CO_2			35	High CO_2			35	High CO_2			35	High CO_2			35	High CO_2			31	High CO_2		
35	43.4			40	42.5			40	41.4			40	44.0			40	40.1			40	41.6			40	43.7			35	39.8		
45	43.2			50	42.3			50	40.4			50	43.7			50	40.9			50	40.9			66	43.2			45	40.0		
58	44.6			65	42.6			65	41.0			65	44.2			65	40.7			65	39.2			76	43.9			57	40.2		
73	43.2	43.6	83.0	74	42.8	42.5	92.0	74	41.7	41.1		74	44.0	43.9	85.0	74	39.6	40.3	85.0	74	40.9	40.6	90.0			43.6	89.0	70	39.9	40.0	83.0
74	Air			75	Air			75	Air			75	Air			75	Air			75	Air			77	Air			72	Air		
78	46.1			80	46.1			80	43.4			80	46.5			80	41.2			80	44.7			84	46.7			75	40.6		
93	45.5			95	46.1	46.1		95	45.0			95	46.9	46.7		95	40.8			95	44.9			99	47.2			90	41.1		
108	45.9	45.8						110	44.3	44.2						110	41.7	41.2		110	44.9	44.8		114	46.2	46.7		105	41.5	41.0	

TABLE 5

The transfer of bicarbonate between the blood and tissues of cats, caused by increasing the carbon dioxide concentration in the lungs

EXPERIMENT 11				EXPERIMENT 12				EXPERIMENT 13				EXPERIMENT 38				EXPERIMENT 40				EXPERIMENT 41				EXPERIMENT 42			
Weight 4.5 kgm.				Weight 4 kgm.				Weight 3.6 kgm.				Weight 5.6 kgm.				Weight 5.5 kgm.				Weight 4.7 kgm.				Weight 3.8 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air				Air				Air				Air				Air				Air				Air		
	vols. per cent		*mm.* Hg		*vols. per cent*		*mm.* Hg		*vols. per cent*		*mm.* Hg		*vols. per cent*		*mm.* Hg		*vols. per cent*		*mm.* Hg		*vols. per cent*		*mm.* Hg		*vols. per cent*		*mm.* Hg
0				0	45.0			0	45.4			0	46.4			0	48.0			0	46.5			0	46.1		
30	45.6	45.6	30.0	21	45.3	45.1	32.0	15	43.5	44.4	33.0	20	46.0	46.2	30.0	20	46.9	47.4	31.0	20	43.5	45.0	38.0	20	44.1	45.1	26.0
32	High CO_2			28	High CO_2			25	High CO_2			30	High CO_2			30	High CO_2			30	High CO_2			30	High CO_2		
45	39.2			43	39.8			30	41.8			35	42.6			35	43.6			35	41.4			35	42.2		
60	38.4			58	40.3			55	41.3			48	41.5			48	42.9			48	42.3			48	42.7		
75	39.4			73	40.5			70	40.7			62	40.7			62	43.1			62	42.1			62	42.0		
90	38.2	38.8	90.0	88	40.7	40.3	91.0			41.2	90.0	74	41.2	41.5	90.0	74	43.7	43.3	92.0	74	41.7	41.8	87.0	74	42.5	42.3	84.0
92	Air			90	Air			80	Air																		
120	46.0			133	43.8			85	45.2																		
140	46.4	46.2		148	43.7	43.7		115	44.2	44.7																	

TABLE 6

The transfer of bicarbonate between the blood and tissues of dogs, caused by diminishing the carbon dioxide concentration in the lungs

EXPERIMENT 44					EXPERIMENT 45					EXPERIMENT 46					EXPERIMENT 47*					EXPERIMENT 48*					EXPERIMENT 56				
Weight 12.2 kgm.					Weight 11.4 kgm.					Weight 12.2 kgm.					Weight 9 kgm.					Weight 8.5 kgm.					Weight 9.9 kgm.				
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure
Air					Air					Air					Air					Air					Air				
	vols. per cent		*mm.* Hg	*mm.* Hg		*vols. per cent*		*mm.* Hg	*mm.* Hg		*vols. per cent*		*mm.* Hg	*mm.* Hg		*vols. per cent*		*mm.* Hg	*mm.* Hg		*vols. per cent*		*mm.* Hg	*mm.* Hg		*vols. per cent*		*mm.* Hg	*mm.* Hg
0	46.6			170	0	41.4				0	41.4				0	48.2			130	0	40.4				0	47.9			
		46.6	46.0		24	41.3	41.3	46.0	150	32	40.9	41.1	35.0	168	25	47.4	47.8	46.0	124	27	41.5	40.9	34.0	144	24	47.2	47.5	46.0	161
25	Overventilation				30	Overventilation				40	Overventilation				45	Overventilation				33	Overventilation				28	Overventilation			
48	44.2			144	37	43.1			100	60	40.7			95	60	45.7			70	41	43.8			109	38	48.2			62
63	45.1				50	42.9			110	70	41.4				69	45.6				55	41.7				48	45.8			
73	45.5				60	42.6				80	42.3			92	85	45.1			85	64	41.9			96	58	46.3			70
83	46.1	45.2	19.0	150	75	42.2	42.7	12.0	100	95	41.4	41.4	8.0	84	96	45.9	45.5	8.0	92	77	40.5	42.0	8.0	100	73	45.7	46.5	9.0	86
85	Air				76	Air				98	Air				100	Air				78	Air				75	Air			
90				166	81	41.3			132	115	39.4			157	110	43.3			126	87	38.4			132	86	46.0			140
					95	41.7			120	135	42.9				125	44.9				107	40.9				103	47.4			
					110	42.2	41.7		120			41.1		154			44.1		124			39.6		135			46.7		142

* Positive pressure pump.

of carbon dioxide to 43.6 volumes per cent. After breathing the carbon dioxide mixture for 43 minutes, room air was substituted and the bicarbonate level of the blood rose again to 45.8 volumes per cent carbon dioxide, only 0.4 volume per cent less than the original level.

In summarizing the average results on dogs as given in table 4, it is found that during the inhalation of a gas mixture containing 11 per cent carbon dioxide (for a period of 40 minutes), the alveolar carbon dioxide tension was raised from 43 to 88 mm. of mercury, and the bicarbonate level of the blood fell from 44.1 to 42.0 volumes per cent of carbon dioxide. The level fell in individual cases from 0.3 to 3.3 volumes per cent carbon dioxide and not a single instance of a rise was observed. During the recovery period on air, immediately following the inhalation of the carbon dioxide gas mixture, the bicarbonate level returned to 44.6 volumes per cent carbon dioxide.

The effect which high alveolar carbon dioxide tensions have upon the bicarbonate level of cats' blood is given in table 5. The level falls from 45.5 to 41.3 volumes per cent carbon dioxide during the inhalation of the carbon dioxide gas mixture, a departure from the normal level which is just twice as great as in the case of dogs.

The effect of lowering the alveolar carbon dioxide tension upon the bicarbonate level of blood. The data are shown in table 6. Only dogs were used. The effect upon the bicarbonate level of reducing the alveolar carbon dioxide tension to 8 mm. by over-ventilation was negligible. In some cases the level was slightly raised and in other cases slightly depressed. The approximation to the normal bicarbonate level which followed the discontinuance of over-ventilation was not as close as that which followed the discontinuance of the carbon dioxide inhalation.

The fall in the blood pressure caused by over-ventilation is undoubtedly due to the lowered carbon dioxide tension throughout the body, as shown by Dale and Evans (1922). An alveolar carbon dioxide tension of about 8 mm. is probably the limit which is consistent with a steady state during over-ventilation and with a normal recovery after the return to natural breathing. The apnea which followed the discontinuance of the artificial respiration was only of short duration, and an approximation to the normal blood pressure followed immediately. It is very apparent that over-ventilation within the limits set by our experiments is in no way prejudicial to a complete and instant return to the normal condition after natural breathing has been reëstablished.

Over-ventilation was administered by the negative pressure method (Shaw and Drinker, 1929) in experiments 44, 45, 46, and 56, and by the positive pressure method through a tracheal cannula in experiments 47 and 48. Since the former method of over-ventilation is free from any interference with the pulmonary circulation or venous return to the heart,

we had hoped by its application to attain low levels of alveolar carbon dioxide unattended by the marked fall in blood pressure which characterizes artificial ventilation by intratracheal insufflation. The blood pressure was affected to the same extent by both methods of over-ventilation. This affords further evidence that the fatal effects of excessive over-ventilation are probably due to lowering of the carbon dioxide tension of the body rather than to the mechanical interference with the circulation.

Discussion. Our work is wholly at variance with that of Henderson and Haggard (1918a). They found that when the alveolar carbon dioxide tension was increased from about 40 mm. tension to 52 mm., as a result of the depressed breathing following morphine administration, the bicarbonate level of the blood was increased by about 12 volumes per cent. During the rebreathing of a carbon dioxide gas mixture from a gas bag, the bicarbonate level of the blood was raised 13 volumes per cent when the concentration of carbon dioxide in the respired air reached 10.7 per cent. In these last experiments the alveolar carbon dioxide tension was probably about 90 mm. and was comparable to the alveolar tension of carbon dioxide attained in our own experiments. When dogs were subjected to passive over-ventilation, on the other hand, Henderson and Haggard (1918b) found that the bicarbonate level was lowered by 12 to 22 volumes per cent of carbon dioxide.

In order to ascertain the possible effect which the barbital sodium might have upon the change in the bicarbonate level caused by the inhalation of carbon dioxide, we performed two experiments upon dogs without the use of barbital sodium. The gas mixture containing 8.3 per cent carbon dioxide was administered from a head mask, and the blood samples were withdrawn from the femoral artery, which had been cannulated under local anesthesia. In one case the bicarbonate level fell 3 volumes per cent and in the other case fell 1.6 volumes per cent following the inhalation of the gas mixture. These results are comparable to those given in table 4. It was also found that the bicarbonate level of blood drawn from the jugular vein of normal intact dogs breathing room air was identical to that of the blood drawn from dogs 3 hours after the intravenous injection of barbital sodium. These experiments make it clear that the bicarbonate level of blood is in no way affected by an intravenous injection of barbital sodium after time has been allowed for equilibrium conditions to become established.

Carbon dioxide absorption curves were constructed for blood taken from dogs while breathing air and compared with the curves for blood taken during the inhalation of the gas mixture containing 10 per cent carbon dioxide. The data are given in table 7. Though the absorption curves of blood taken during the inhalation of carbon dioxide are not in all cases parallel to those made from normal blood, the former are, in every case

and at all points, lower than the latter. Though the buffering qualities of the blood subjected in vivo to high tensions of carbon dioxide appear to have been somewhat altered from the normal, in no instance do the curves cross, thereby converting an apparent fall of the bicarbonate level as estimated at a given carbon dioxide tension into a rise when estimated at a different tension. There is a lowering of the bicarbonate level of blood taken during the inhalation of carbon dioxide at all carbon dioxide tensions.

The readiness with which sodium bicarbonate passes from the blood into the tissue fluid, and presumably in the reverse direction under appro-

TABLE 7

The effect of high alveolar carbon dioxide tensions upon the carbon dioxide absorption curve of dogs' blood

EXPERIMENT	20 MM.			40 MM.			60 MM.		
	Air	CO_2	Difference	Air	CO_2	Difference	Air	CO_2	Difference
20	30.3	26.0	4.3	42.2	39.3	2.9	50.4	47.1	3.3
21	29.1	29.4	0.3	43.5	41.9	1.6	53.4	50.5	2.9
23	31.0	26.0	5.0	42.3	39.4	2.9	48.0	46.0	2.0
24	26.1	23.0	3.1	34.0	32.5	1.5	42.0	36.6	5.4

TABLE 8

The per cent of sodium bicarbonate which is retained by the plasma 30 minutes after its injection intravenously

EXPERIMENT	PLASMA VOLUME	VOLUME CARBON DIOXIDE INJECTED	VOLUME CARBON DIOXIDE RETAINED BY PLASMA AFTER 30 MINUTES	PER CENT RETAINED
	cc.	*cc.*	*cc.*	*per cent*
1	107	267	23.3	8.7
2	103	280	25.5	9.1
3	103	259	31.0	12.0
4	128	292	21.8	7.5
5	128	289	26.9	9.3

priate conditions, can be shown by a very simple experiment. About 50 cc. of an 0.268 molar solution of sodium bicarbonate were injected slowly into the femoral vein of a cat. Except for the small quantity of sodium bicarbonate accumulating in the pelvis of the kidney, escape via the kidney was prevented by the ligation of the ureters. At 15-minute intervals blood samples were taken under oil and the plasma, which was allowed to separate from the whole blood while resting over mercury in a blood collecting tube, was analyzed for carbon dioxide content. The total volume of plasma in the cat was calculated on the basis that the blood volume was 5.5 per cent of the body weight and the plasma 65 per cent of the whole blood. The amount of bicarbonate which was retained by the plasma

at the end of a given time following the injection was determined by the departure from the normal carbon dioxide content of the blood. Table 8 gives the data for five experiments. Speaking in terms of averages it shows that, following an injection of sodium bicarbonate solution carrying 277 cc. of carbon dioxide, only 25.7 cc., or 9.3 per cent of the original injection, were left in the plasma at the end of 30 minutes.

It follows from these considerations that whenever the bicarbonate level of the plasma is raised above that of the tissue fluids, bicarbonate will leave the blood; and conversely, when the bicarbonate level of the plasma is depressed below that of the tissue fluids, bicarbonate will flow into the blood. In order to eliminate confusion we may safely overlook the slight difference which may exist under conditions of equilibrium between the bicarbonate level of the plasma and tissue fluids caused by the Donnan equilibrium.

The bicarbonate of the plasma is due to the reaction of carbonic acid with the alkali salts of the protein and with the chlorides according to the following equations:

(1) $H_2CO_3 + \text{Na Proteinate} = NaHCO_3 + \text{H Proteinate}$

(2) $H_2CO_3 + NaCl = NaHCO_3 + HCl$

In equation 1, sodium proteinate is used to indicate the alkali salts of the plasma proteins, and the hydrogen proteinate the acid proteins. The hydrochloric acid in equation 2 will pass into the blood cells leaving one molecule of sodium bicarbonate in the plasma for every molecule of hydrochloric acid bound by the alkali buffers of the blood cells. The equilibria as written will shift from left to right with an increase of carbonic acid and cause an increase of bicarbonate. As the carbonic acid decreases, the equilibria will shift from right to left and the bicarbonate will be diminished.

In explanation of the migration of bicarbonate out of the blood we offer the following hypothesis. When the carbon dioxide tension in the lungs is raised, the bicarbonate content of the blood is increased, followed immediately by an increase in the bicarbonate content of the tissue fluids. If the carbon dioxide combining capacity of the tissue fluids were exactly equal to that of the blood then the bicarbonate level in the two systems of fluid would remain equal. Under these conditions no transfer of bicarbonate between the blood and tissue fluids would take place. On the other hand, if the carbon dioxide combining capacity of the blood were greater than that of the tissue fluids, then an increase in the carbon dioxide tension of the lungs would cause the formation of more bicarbonate in the blood than in the tissue fluids, and the bicarbonate level in the blood would rise above the level attained in the tissue fluids. Under these conditions bicarbonate would move out of the blood into the tissue fluids until equilibrium is again established.

The work of Shaw and Messer (1930) upon the carbon dioxide combining

capacity of the tissue fluids gives support to the above hypothesis. They made cats breathe a gas mixture containing about 11 per cent carbon dioxide for a sufficient time to bring the entire body into equilibrium with the increased carbon dioxide tension of the alveolar air. Outdoor air was then substituted for the gas mixture and the expired air was collected during the period of desaturation. The carbon dioxide that was retained by the body, and subsequently eliminated in response to a return of the normal alveolar carbon dioxide tension, could then be evaluated by subtracting the metabolic carbon dioxide from the total carbon dioxide. Since the carbon dioxide retained by the whole body is held in the fluids of the body, the carbon dioxide taken up by the tissue fluids is equal to the carbon dioxide retained by the whole body minus the carbon dioxide taken up by the blood. Shaw and Messer found that when the carbon dioxide tension was raised from 30 mm. to 90 mm. of mercury, 1 kilo of cats' blood combined with 3.2 cc. of carbon dioxide per millimeter of carbon dioxide tension difference, and that 1 kilo of tissue fluid combined with 2.5 cc. per millimeter of carbon dioxide tension difference. The tissue fluids, therefore, have a carbon dioxide combining capacity which is 78 per cent as great as that of blood.

Since the buffer value of the tissue fluids of the cat is only 78 per cent of the blood capacity, it follows that there will be 22 per cent less bicarbonate formed in the tissue fluid than in the blood, following an increased carbon dioxide tension in the lungs. As a consequence the blood should lose 22 per cent of its bicarbonate in order that equilibrium may be established between the two systems of fluid. The bicarbonate level of the tissue fluid will not be appreciably affected by this transfer since the volume of the tissue fluid is at least ten times as great as the volume of blood, so that approximately nine-tenths of the adjustment takes place in the blood.

In our experiments on cats, the alveolar carbon dioxide tension was raised from 33 mm. to 90 mm., so that the blood absorbed about 18.2 volumes per cent of carbon dioxide (57×0.32 volumes per cent). We have seen that about 4.2 volumes per cent of carbon dioxide were lost. This is equivalent to a 23 per cent $\left(\frac{4.2}{18.2}\right)$ loss of the blood bicarbonate. Thus the estimated loss of bicarbonate (22 per cent) from the blood, based upon the relative buffering value of blood and tissue fluid, is in striking agreement with the observed loss (23 per cent).

When there is no migration of bicarbonate into or out of the blood we may assume that the buffer values of the blood and tissue fluids are equal. When there is a migration of bicarbonate it signifies that the buffer value of the blood is either greater or less than that of the tissue fluids, and that bicarbonate is moving in the direction of its lowest concentration. In the

case of the dog, the migration of bicarbonate takes place very slightly in both directions when the carbon dioxide tension has been lowered, indicating that at tensions below normal the carbon dioxide absorption curve of the tissue fluids is essentially coincident with that of the blood. When the carbon dioxide tension has been raised, bicarbonate migrates out of the blood, indicating that at some tension above the normal the carbon dioxide absorption curve of the tissue fluids falls below the blood curve and becomes somewhat flatter.

Following a rise in the carbon dioxide tension of the lungs, the migration of bicarbonate into the tissue fluids must take place very rapidly. Since the first sample of blood was taken 5 minutes after the inhalation of carbon dioxide commenced, and was in substantial agreement with samples taken during the next 35 minutes, it is certain that the transfer of bicarbonate was complete in less than 5 minutes.

One might expect that the immediate effect of increasing the bicarbonate concentration of the blood before the concentration in the tissue fluids had been increased, as must be the case when carbon dioxide is inspired, would be a temporary but very marked overflow of bicarbonate into the tissue fluids with a subsequent return of bicarbonate to the blood as the reaction takes place throughout the body. Shaw and Messer (1930) have shown that the body as a whole takes about 90 minutes to come into equilibrium with changes in the carbon dioxide tension of the blood. That equilibrium between the bicarbonate of the blood and the body fluids is established so promptly and is not conditioned by the reaction time of the entire body, is probably due to the fact that carbon dioxide diffuses through tissues more rapidly than bicarbonate, and therefore the bicarbonate concentration of the tissue fluids in immediate contact with the blood is raised to its equilibrium value before the bicarbonate from the blood has passed through the capillary walls. Though it may require approximately 90 minutes for the bicarbonate concentration of the tissue fluid of the entire body to attain its maximum concentration following an increased carbon dioxide tension in the lungs, the concentration of the bicarbonate in the tissue fluid in direct contact with the capillary walls will attain its maximum concentration almost immediately, and thereby establish the equilibrium level for the bicarbonate of the blood.

SUMMARY

1. When the alveolar carbon dioxide tension was raised to about 88 mm. by the inhalation of air enriched with carbon dioxide, the carbon dioxide combining capacity of the blood was lowered by 2.1 volumes per cent in dogs and by 4.2 volumes per cent in cats, as a result of the migration of bicarbonate into the tissue fluid. The time required to bring about equilibrium conditions was less than 5 minutes.

2. When the alveolar carbon dioxide tension of dogs was lowered to about 8 mm., the carbon dioxide combining capacity of the blood was increased in some cases by the migration of bicarbonate into the blood, and was diminished in other cases by the migration of bicarbonate out of the blood, the average change being 1.4 volumes per cent carbon dioxide.

3. An hypothesis, based upon the relative carbon dioxide combining capacity of the blood and the tissue fluids, is offered to explain the transfer of bicarbonate between the blood and the tissues.

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THE EFFECT OF THE INJECTION OF HYPOPHYSEAL EXTRACT IN ADVANCED LACTATION

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During the past two years our conception of the stimuli which are responsible for mammary development has undergone a profound alteration. It has been held as a result of the pioneer work in this field that the stimulus is due entirely to the action of the corpus luteum. But the evidence although it seemed overwhelming, was circumstantial in character and under the fire of recent experimental evidence the interpretation, justifiable at the time, placed upon the results of these pioneers has been thrown open to doubt. In 1929 Grueter and Stricker announced that they had been able to induce full mammary development in the ovariectomised mature rabbit by the injection of an alkaline extract of the anterior pituitary. They found further that the injections were without effect in the case of immature animals, and from an examination of the ovaries of their rabbits concluded that the mammary gland requires a preliminary sensitization by the action of the rabbit's own corpus luteum. Corner (1930), using an alkaline extract of sheeps' whole pituitary, obtained full mammary development in mature virgin rabbits which had been ovariectomised. As examination of the ovaries of the operated animals showed that ovulation had never occurred, it is evident that previous action of the corpus luteum is unnecessary. In addition Corner found that the corpus luteum extract from the sow which would maintain pregnancy in the absence of the rabbit's ovaries is without effect upon the mammary gland when administered to the non-pregnant doe.

Later, Asdell, using the same neutralised alkaline extract of sheeps' whole pituitary supplied by Parke, Davis & Company, was able to confirm the production of full mammary development similar to that of pregnancy. His ovariectomised rabbits were just mature, but careful examination of the ovaries showed that ovulation and consequent formation of corpora lutea had never occurred. It was also found that the potent extracts are without effect in the immature rabbit. It is evident, therefore, that some growth or sensitization is needed before the hypophyseal preparation can become effective. The results were extended to

a goat as daily subcutaneous injection of 10 cc. of the extract (3.35 cc. = 1 gm. whole pituitary) into a young female goat which, so far as was known, had not been in heat resulted in sufficient mammary development to be recognized by a slight increase in the size of the udder while it was possible to express a few drops of milk from the teats. This had been impossible in repeated trials before the injections. The possible stimulating action of handling the gland was guarded against as the udder was not touched for the fortnight during which injections were in progress. Injection of 40 cc. daily into a mature goat, whose lactation period was nearly spent, failed. The extract as prepared at present is very toxic and the goat became emaciated and refused to feed. The milk yield declined. A preliminary report of these experiments has been made (Asdell, 1931).

As the preliminary work had shown that the extract is probably effective in the goat, an attempt was made this year to determine whether continued administration of the extract to the mature lactating goat would prevent the normal decline in lactation by maintaining the growth of the mammary gland at its normal maximum or beyond it. Messrs. Parke, Davis Company again generously supplied the considerable quantity of extract which was necessary for this work. Each batch was freshly made and despatched by express at regular intervals. It was kept in an ice chamber during the experiment and tested by injection into ovariectomised rabbits. It proved to be potent. The method of preparation and strength were as before. The dose used to test the effect of the extract on rabbits was 3 cc. per day for 8 days. Three goats were used in the main experiment and the injections arranged as follows:

Goat D. Date of parturition, January 29, yield at time of experiment, June 1, 1700 cc. daily. Injected subcutaneously with 10 cc. after the evening milking.

Goat B. Date of parturition, January 29; milk yield 1700 cc. Daily injection 20 cc.

Goat C. Date of parturition, February 4; milk yield 1600 cc. Daily injection 30 cc. from June 12 to July 2 when injection was dropped to 10 cc. daily as the goat began to show ill effects from the injections.

Three control goats A, E and F, dates of parturition February 24, January 29, and February 21 respectively, received no injections but were kept in the same room under similar conditions. All goats were milked at 8:30 a.m. and 4:00 p.m.

The injections commenced on June 12th and continued until July 24th.

Goat B was considerably older than the others and she soon refused to feed and became emaciated. Her milk yield dropped considerably and she was discarded. The toxicity of the extract is considerable and she evidently felt the ill effect. The yield of the other two goats increased for a period of 36 days but they then refused their feed and lost weight. The milk yield consequently declined rapidly. This ill effect was probably due to the accumulated toxicity of the extract. The use of a new batch

of extract three days after the decline in milk yield began did not improve the yield.

It is to be noted that the milk yields of the injected goats were increasing at the time when those of the controls showed a marked falling off. This is the more remarkable as it includes a period of exceptionally hot

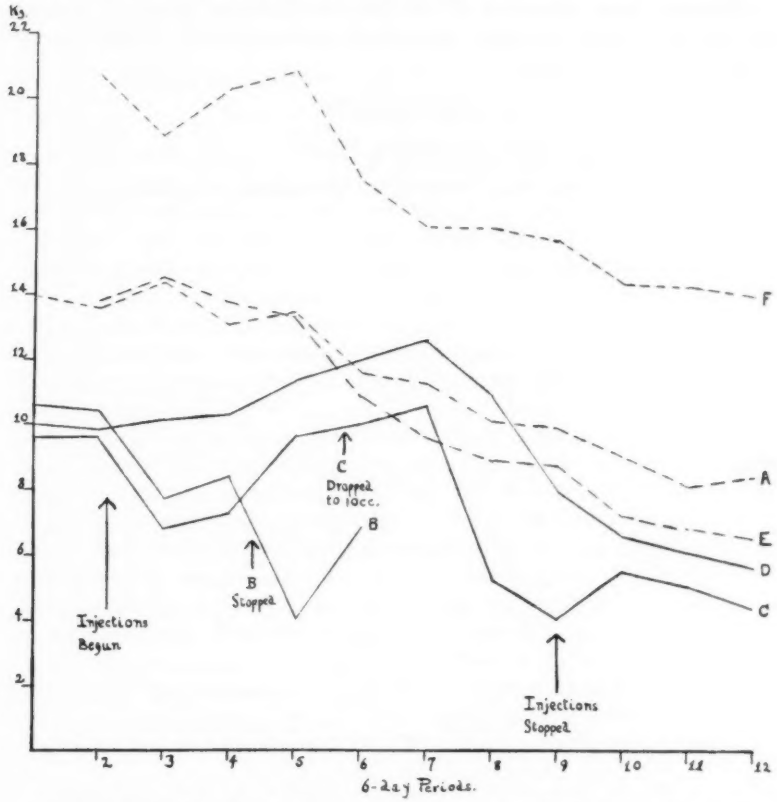

Fig. 1. Anterior hypothesis and milk yield in goats. Ordinates: milk yield in kilograms by 6-day periods. Abscissae: 6-day periods. Controls: broken lines. Injected goats: continuous lines. Daily injections: Goat D, 10 cc. Goat B, 20 cc. Goat C, 30 cc. at first, then 10 cc.

weather. In the graph, the daily milk yields have been added and the quantity yielded in six day periods reported. We feel that the conclusion is justifiable that the extract of anterior lobe is effective in preventing, for a time at any rate, the normal decline in milk yield with advancing lactation. Whether the gland can be built up further than is normal for

the animal, and whether the total milk yield for the lactation period can be increased remains to be determined in future work with a less toxic preparation.

SUMMARY

Repeated daily injections of alkaline extract from sheep hypophyses prevent, for a time at least, the normal decline of milk yield in goats with advancing lactation.

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RELATION OF PROLAN TO THE ANTERIOR HYPOPHYSEAL HORMONES[1]

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Aside from its value as a test for pregnancy, the discovery of a gonad-stimulating hormone in the urine of pregnant women by Aschheim and Zondek has acquainted us with a new fact of the deepest interest to those concerned with the physiology of reproduction. The source of this hormone is still unknown to us, though it has been generally assumed that it comes from the hypophysis. Its effect on the ovary of an infantile animal resembles strikingly the effect produced by "implants" of anterior hypophyseal tissue and of no other tissue known to us save the placenta. It was hence natural to assume that this urinary constituent originates in the hypophysis.

As this laboratory has been concerned for some years with hypophyseal hormones, we early instituted comparisons between the gonad-stimulating hormone from the urine and that which can be extracted from the hypophysis.[2] There are some striking differences between these substances. When prolan is administered in increasing doses, the response of the ovary corresponds to the dosage only up to a certain limit. When doses are given exceeding this limit, no matter what the dose or how often it is administered, the effect on the ovaries is practically the same. If, on the other hand, one gives increasing dosage either in the form of implants of hypophysis, or of extracts of hypophysis, the weights of the ovaries produced increase with the dosage. As an example of the effect of increase in implant dosage we cite an experiment from a previous paper.[3]

A single implantation of half of a rat's anterior lobe caused fifty per cent of the animals to mature and the average ovary weight was 21 mgm. With all higher im-

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, and from the Rockefeller Foundation. These funds have been generously augmented by the Board of Research and the College of Agriculture of this University.

[2] For convenience in this paper we will use Zondek's term "prolan" to designate the hormone from urine. By "gonad-stimulating" hormone we do not necessarily imply a single substance. We do not wish to discuss the problem of multiplicity of gonad-stimulating hormones in the hypophysis at this time.

[3] Evans, H. M. and M. E. Simpson. This Journal, 1929, lxxxix, 381.

plant "dosage" practically all animals matured in 100 hours and the ovary weights varied with the dose level as follows: a dose of one anterior lobe gave ovaries weighing 68 mgm.; 4 lobes gave ovaries weighing 105 mgm.

In the following chart (fig. 1) the effect of increasing the dosage of extracts from the anterior hypophysis is contrasted with the effect of increasing the dosage of prolan. The response of the ovary of the immature test animal to different dose levels of these substances was measured by the weight of ovaries produced within 100 hours. It can be seen that

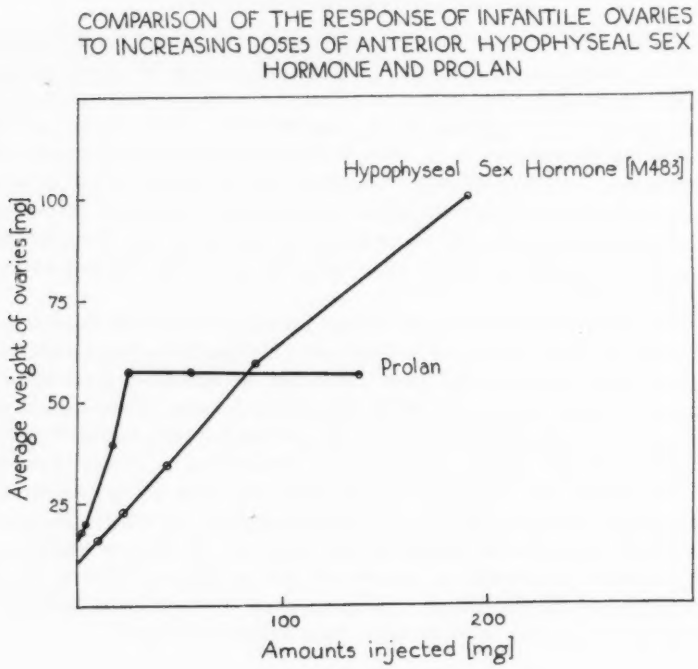

Fig. 1

in the case of prolan administration, the curve in which ovary weight is plotted against dose level quickly reaches a plateau and that a further fivefold or even tenfold increase in dose does not cause a further increase in ovary weight. In the case of administration of the anterior hypophyseal gonad-stimulating hormone, the curve expressing the relation between dose and ovary weight continues a steep ascent as higher dose levels are reached. It has been possible by injection of hypophyseal sex hormone to provoke ovaries weighing up to 190 mgm. within 100 hours. On the other

hand, it is seldom possible even with 200 or more units[4] of prolan to stimulate the production of ovaries weighing over 70 mgm. in this time interval. In cases of administration of other preparations of the hypophyseal sex hormone we have found the ascent of the curve to be even steeper than in the case recorded here. All of our extracts of the gonad-stimulating hormone from the hypophysis itself whether crude or purified were alike in showing this type of curve.

Perhaps the most important reason for assuming that prolan and the hypophyseal gonad-stimulating hormone are different is the relative inefficacy of prolan in hypophysectomized animals as reported in the preceding paper of this series. In contrast to these findings it has been shown by Smith, Reichert and others that the infantile reproductive systems of such hypophysectomized animals can be stimulated to complete development and function by the administration of anterior hypophyseal material.[5]

Briefly then, though prolan and anterior hypophyseal hormone appear to have the same qualitative effect on the ovary of the normal infantile animal, they are dissimilar in that prolan is embarassed in its action in the hypophysectomized animal and is limited in the effects it can provoke in the normal infantile animal. On the other hand the gonad-stimulating anterior hypophyseal hormone is effective in the hypophysectomized animal, and does not suffer the curious limitation of its effect on the ovaries of the normal immature animal. We must assume, it would appear, that the hypophysis itself plays a rôle in the action of prolan. Since prolan appears to have the same qualitative effect on the ovary as has the hypophyseal hormone, it would seem that we must think of prolan either as *provoking the production of the gonad-stimulating hormone of the hypophysis, or as converting some inactive component in the hypophysis into an active substance.* The limited effect of prolan on the normal infantile ovary would be clear for it could be considered as a direct consequence of low content or low production of the mother substance in the hypophysis ofthe immature animal. Even in the sexually mature animal the amount of active substance that can be demonstrated by the implantation method or by extraction is surprisingly low, especially in beef.

Since we can extract directly from the hypophysis the gonad-stimulating hormone, we must assume, if the previously expressed theory is correct, either that this amount of hormone had been previously activated within

[4] The unit of prolan to which reference is made is the minimum dose which administered in three daily subcutaneous doses to immature rats produces in 96 hours the development of ovaries containing a corpus or corpora. Only one of the three test animals needs respond.

[5] Smith, P. E. The disabilities caused by hypophysectomy and their repair. Journ. Amer. Med. Assoc., 1927, lxxxviii, 158. Reichert, F. L., The results of replacement therapy in an hypophysectomized puppy: four months of treatment with daily pituitary heterotransplants. Endocrinol., 1928, xii, 451.

the hypophysis by a prolan-like substance; or that it had been activated by a substance freed during preparation, or finally, that the inert substance or prohormone had been activated at the site of injection. The assumption that an activator is present in the hypophysis itself seems more probable to us.

A direct proof of this theory of the activation of an inert substance in the hypophysis would be furnished if by adding the activator to a given preparation from the hypophysis we could produce effects not secured from the hypophyseal preparation alone. The experimental confirmation of the theory was surprisingly satisfactory. The combination of prolan and hypophyseal extract gave a far greater effect than could be obtained from the administration of either component alone or than could be expected on the basis of an additive effect.

EXPERIMENTAL. The procedure was as follows: groups of three animals 24–26 days of age were injected daily, subcutaneously, for three successive days (Monday, Tuesday, Wednesday) with 1 cc. doses of the different aqueous preparations. The animals were observed twice daily after the 50th hour (Wednesday) for rupture of the vaginal membrane. After 96 hours, i.e., on the fourth day (Friday), the animals were sacrificed and the genital tract was examined under a binocular microscope. The ovaries were dissected free of bursa and oviduct and weighed on an analytical balance to an accuracy of 1 mgm. The size and vascularity of the genital systems and in particular the size and number of follicles and corpora and the presence of blood points were noted. The ovaries were sectioned when occasion demanded.

The methods of preparation of extracts from hypophysis and urine will be reported in detail in another paper. We are concerned in this paper with more purely biological investigations. The source of the hormone from the urine of pregnant women used by us was a crude prolan kindly furnished to us by the I. G. (Elberfeld)[6]. The crude prolan powder was extracted with acidulated water and poured into alcohol. The precipitate was washed with alcohol and ether and quickly dried in vacuo. The minimal potent dose of this powder when redissolved in water was 1.5 mgm. (0.5 mgm. daily for 3 days). Beef hypophyses were the source of the anterior lobes used by us in the preparation of the hypophyseal extracts reported here. Stable dry powders were prepared by acetone precipitation and were used in most of the experiments reported in this paper. Unless otherwise stated, they contained both the gonad-stimulating and the growth hormone. Other more purified preparations in which growth and sex hormones had been separated were also used and will be indicated in the appropriate place in the text and tables. All preparations were brought to neutrality (pH 7.4) before injection.

[6] In particular we wish to thank Dr. Werner Schulemann and Dr. Fritz Lacquer.

TABLE 1

Effects on the ovary of the immature rat of prolan and hypophyseal hormones given separately and in combination

	DESIGNATION OF EXPERIMENT															
	486	502	509	307	332	308	328	329	456	502	459	429	431	430	419	426	421
Dose* prolan, mgm.	54.2	0	54.2 (#486)	136.3	0	136.3 (#307)	54.5	54.5 (#328)	54.5	0	54.5 (#456)	136.3	0	136.3 (#429)	13.6	0	13.6 (#419)
Dose A. H. H., mgm.	0	65.0	65.0 (#502)	0	65.0	65.0 (#332)	0	65.0 (#332)	0	65.0	65.0 (#502)	0	65.0	65.0 (#431)	0	65.0	65.0 (#426)
Average weight of ovaries, mgm.	62.0	21.3	156.3	59.7	22.7	131.3	59.3	136.3	60.0	21.3	117.0	56.7	21.3	117.7	37.3	26.7	74.7
Activation			151%			120%		121%			95%			107%			100%

* By "dose" we mean the weight in mgm. of dried material dissolved in 3 cc. of water and administered to each animal in 1 cc. doses for 3 days.

Examples of activation of hypophyseal preparations by prolan. In table 1 we present examples of the effect on the immature ovary of combining prolan with hypophyseal preparations. Given amounts of prolan and hypophyseal preparations were administered, separately and after combination. The combination was made in vitro. The total volume was equal to that of the individual constituents when injected alone. The dose levels of hypophyseal preparations were chosen carefully so that they were barely able to provoke precocious maturity (with corpora formation) or fell just below the minimum dose level. The prolan preparations were usually administered at a dose level at which they already provoked the maximum effect possible with prolan. They stimulated numerous corpora lutea, large follicles and follicular cysts. It can be seen in the table that the injection of the combination of these constituents, administered at these dose levels, stimulated the development of ovaries weighing far more than the ovaries provoked by the individual constituents alone—also larger ovaries than could be expected from any additive effect resulting from the combination of the two constituents. The ovaries stimulated were usually characterized by great numbers of corpora though large follicles and follicular cysts were often present.

The percentage activation was calculated as the relation between ovary weight stimulated by prolan and the increased weight due to the combination. The ovary weight of the hypophysis-injected rats was neglected in our calculation of activation because the weight of these ovaries was seldom more than the weight of infantile ovaries.

Specificity of the activation. We next wish to give the evidence for considering that the activation of hypophyseal preparations is a specific reaction. We tested the specificity of this reaction in three ways: first, we combined prolan with active and inactivated hypophyseal hormone; secondly, we combined prolan with extracts from other organs prepared in a manner identical with that used in preparing hypophyseal extracts; thirdly, we substituted for prolan extracts from tissues prepared in the same way prolan is prepared.

The hypophyseal hormone was inactivated by being heated for ten minutes in vigorously boiling water, or for 30 minutes at 70–80°C. The hypophyseal hormone heated for 30 minutes at 70–80°C., when administered in combination with prolan, no longer produced large ovaries but only those of the weight provoked by prolan alone.[7] The shorter treatment at 100°C. apparently did not completely inactivate the hypophyseal component so that the result of combination of the heated product with prolan while less than that in cases where active hormone was used (see

[7] Since the visible physical properties of the hypophyseal hormone preparation when heated to 70–80°C. were unchanged, the probability that the activation phenomenon is due to some physical and hence non-specific effect is excluded.

table 2 A) was still greater than the effect of administration of prolan alone.

The second method of testing the specificity of the activation was as follows: calf and pig liver were extracted and treated in a manner similar

TABLE 2A

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (A, Effect of the destruction of the specific substances by heat)

	DESIGNATION OF EXPERIMENT								
	328	332	329	334	330	307	308	310	309
Dose prolan, mgm.	54.5 (*307)	0	54.5 (*307)	0	54.5 (*307)	136.3	136.3 (*307)	0	136.3 (*307)
Dose A. H. H., mgm.	0	65.0 (Active solution)	65.0 (Active solution *332)	65.0 (Inactivated 34′ at 70–80° C. *332)	65.0 (Inactivated 34′ at 70–80° C. *332)	0	65.0 (Active solution *332)	65.0 (Inactivated 10′ at 100° C. *332)	65.0 (Inactivated 10′ at 100° C. *332)
Average weight of ovaries, mgm.	59.3	22.7	136.3	13.7	62.3	59.7	131.3	16.7	79.0
Activation			121%		None		120%		Inconsiderable

TABLE 2B

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (B, Substitution of a non-specific substance, liver, for the hypophyseal component)

	DESIGNATION OF EXPERIMENT						
	486	505	503	509 (control)	546	549	556
3 days prolan dosage, mgm.	54.5	0	54.5 (*486)	54.5 (*486)	54.5	0	54.5 (*546)
3 days liver dosage, mgm.	0	54.5	54.5 (*505)	65.0 (A. H. H. *502)	0	65.0	65.0 (*549)
Average weight of ovaries, mgm.	62.3	17.3	55.3	156.3	53.7	17.0	47.5
Activation			None	150%			None

to that used in making hypophyseal preparations. This product was administered separately and in combination with prolan as in the usual procedure. In table 2 B it can be seen that the potency of the prolan was not increased by combination with the liver preparations. But the

potency of the same prolan was increased when combined with a hypophyseal preparation. This is shown in the table for contrast.

It would seem from the above data that the hypophyseal component of our combination is certainly a specific one. We have endeavored to examine similarly the specificity of prolan. Since the activation we describe resembles a ferment reaction, we tried to substitute certain ferments in place of prolan. We chose liver as the source of the ferment partly because the liver is well known to be rich in these substances, but chiefly because we suspected that the reaction described in this paper might

TABLE 2C

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (C, Substitution of a non-specific substance for the prolan)

	DESIGNATION OF EXPERIMENT								
	505	502	504	506	549	555	548	550	551
3 days dose liver, mgm.....	54.5 (Pig)	0	54.5 (*505)	27.2 (*505)	54.5 (Calf)	0	54.5 (*549)	54.5 (*549)	54.5 (*549)
3 days dose A. H. H., mgm.....	0	65.0	65.0 (*502)	65.0 (*502)	0	65.0	65.0 (*555)	65.0 (*555) 1 hr. at 37°C.	65.0 (*555) 1 hr. at 37°C.
Average weight of ovaries, mgm.....	17.3	21.3	35.7	28.3	17.0	30.7	37.7	42.7	34.7
Activation..			69%	33%			Inconsiderable	39%	Inconsiderable

bear a relation to the arginine content of the hypophysis. In a later paper we will elaborate on the reasons which led to these deductions. In table 2 C we give some examples showing the effect of administering a liver powder in combination with hypophyseal extracts. Although there was a slight difference in the effects of administering the hypophyseal preparation alone and in combination with liver, the difference may not be significant. To establish such a nonspecific activation would naturally require much further biological and chemical investigation.

Quantitative relations. We have considered two possible mechanisms

by which the activation process may take place. Either a stoichiometric combination occurs between the two components or the conversion of a mother substance, a prohormone, is facilitated by a catalyst. In the first case we should expect the reaction to involve definite proportions of the reacting constituents. If the second supposition were true, we should expect the reaction to be more nearly independent of one of the constituents, namely, the catalyst. We tried to decide between these possibilities by experiments in which we varied the amounts of each constituent, hypophysis and prolan. When we administered constant amounts of prolan

TABLE 3

Effect on ovaries of immature rats of combining different amounts of hypophyseal preparations with a constant prolan

	DESIGNATION OF EXPERIMENT								
	375	220	446	450	328	332	329	333	331
3 days prolan dosage, mgm..	27.2	0	27.2 (*375)	27.2 (*375)	54.5	0	54.5 (*328)	0	54.5 (*328)
3 days A. H. H. dosage, mgm.....	0	112.5 (sex free growth hormone)	45.0 (*220)	22.5 (*220)	0	65.0	65.0 (*332)	6.5 (*332)	6.5 (*332)
Average weight of ovaries, mgm.....	32.7	21.0	85.1	53.0	59.3	22.7	136.3	17.7	60.3
Activation..			160%	62%			121%		None

with decreasing amounts of hypophyseal hormone the ovarian response diminished rapidly (table 3). When on the other hand the hypophyseal component was kept constant and the amounts of prolan were varied the activation values were quite constant after a certain concentration of prolan was reached. The results speak rather for the catalytic theory. When smaller amounts of prolan are used in the reaction there is a considerable decrease in the percentage activation as well as lower absolute values for the ovary weights (table 4).

Constancy of the activation reaction. In tables 5 and 6 we have given the results of several dozen experiments made in an effort to see whether

Effect on the ovary of he immature rat of the combination of

	307	332	308	328	329	429	431	430	369	374	376
3 days dose prolan, mgm.	136.3	0	136.3 (#307)	54.5 (#307)	54.5 (#307)	136.3	0	136.3 (#429)	54.5 (#429)	54.5 (#429)	27.2 #429
3 days dose A. H. H., mgm.	0	65.0	65.0 (#332)	0	65.0 (#332)	0	65.0	65.0 (#431)	0	65.0 (#431)	65.0 #431
Average weight of ovaries, mgm.	59.7	22.7	131.3	59.3	136.3	56.7	21.3	117.7	52.0	96.0	63.7
Activation			120%		121%			107%		85%	95%

the activation reaction was one which could be duplicated quantitatively. This did not prove to be the case. In table 5 are summarized all results from experiments in which samples from the same prolan preparation were combined with a variety of hypophyseal preparations. In table 6 experiments are cited in which samples from a single acetone powder are combined with different prolan preparations. As can be seen from average

TABLE 5

Effect on the ovary of the immature rat of combinations of different hypophyseal preparations with the same prolan

	DESIGNATION OF EXPERIMENT							
	509	459	374	547	581	376	382	384
Dose prolan #108, mgm.	54.5	54.5	54.5	54.5	54.5	27.2	27.2	27.2
Dose A. H. H., mgm.	65.0 (#120)	65.0 (#120)	65.0 (#107)	65.0 (#16)	65.0 (#120)	65.0 (#107)	65.0 (#97)	65.0 (#93)
Average weight of ovaries, mgm.	156.3	117.0	96.0	88.5	110.0	63.7	63.0	48.3
Activation	150%	95%	85%	65%	77%	95%	84%	48%

ovary weights and from percentages of activation, the results in both sets of experiments varied very considerably. We are aware of the fact that much of this variability can be traced to the small number (three) of animals in our experimental groups.

Component in the hypophysis activated. We have been able to resolve the crude acetone powders of hypophyseal substance employed in these

TABLE 4

nation of ...rent amounts of prolan with a constant hypophyseal preparation

	DESIGNATION OF EXPERIMENT														
374	376	377	378	379	380	427	428	418	420	419	421	422	424	423	425
54.5 (♀429)	27.2 (♀429)	13.6 (♀429)	13.6 (♀429)	2.7 (♀429)	2.7 (♀429)	136.3	136.3 (♀427)	27.2 (♀427)	27.2 (♀427)	13.1 (♀427)	27.2	27.2	27.2 (♀422)	13.1 (♀422)	13.1 (♀422)
65.0 (♀431)	65.0 (♀431)	0	65.0 (♀431)	0	65.0 (♀431)	0	65.0 (♀431)	0	65.0 (♀431)	0	65.0 (♀431)	0	65.0 (♀431)	0	65.0 (♀431)
90.0	63.7	32.3	49.3	19.0	27.0	64.0	114.0	44.3	78.7	37.3	74.7	39.0	72.3	34.7	65.0
85%	69%		53%		38%		78%		79%		100%		85%		91%

studies into chemically and physiologically different substances, into the growth hormone and the gonad-stimulating hormone. We have prepared from the acetone powder, growth hormone free of the gonad-stimulating hormone, also the gonad-stimulating hormone free of the growth promoting factor.[8] We were naturally interested to know whether one or both of these two components of the acetone powder was responsible for the activation. Combinations of prolan with the gonad-stimulating hormone of the hypophysis gave no activation whatever (table 7). On the other hand a marked activation resulted in most cases in which prolan was combined with purified growth fractions (table 8). We have proven that our growth hormone preparations are free of gonad-stimulating hormone but we can not naturally be equally certain that other physiologically active substances are absent. Therefore we can not be sure at present whether some unknown substance is concerned. Nevertheless good grounds exist for the belief that the activated substance is the growth hormone. In the first place our various growth preparations were prepared in radically different ways. Secondly, although not crystalline, they are quite pure as judged by the fact that we have found them to be almost free from serum proteins.[9] Further reason for considering that it is indeed the growth hormone itself which is the activated substance is furnished by the following facts. Slight changes in methods of preparation can be made, which though not radical ones, are sufficient to cause destruction of the growth hormone. These slight changes in procedure are also followed by failure of the activation reaction on combination with prolan.

DISCUSSION. It is necessary to bear in mind the following facts which have been ascertained: Prolan does not easily affect the ovary of the

[8] The purification was tested in each case by the largest quantities that could be administered—the limiting factors being solubility and the amount the animal could tolerate.

[9] Mr. W. R. Lyons has performed precipitin tests on these preparations and has shown that no more than 2 per cent of the total protein is beef serum protein.

TABLE 6

Effect on the ovary of the immature rat of combination of different prolan preparations with the same hypophyseal preparation

	DESIGNATION OF EXPERIMENT												
	509	459	581	582	438	428	430	420	424	378	421	425	378
Dose prolan, mgm.	54.5 (#108)	54.5 (#108)	54.5 (#108)	54.5 (#23)	54.5 (#1)	136.3 (#1)	136.3 (#108)	27.5 (#1)	27.5 (#11)	27.5 (#108)	13.6 (#1)	13.6 (#11)	13.6 (#108)
Dose A. H. H., mgm.	65.0 (#120)	65.0 (#120)	65.0 (#120	65.0 (#120)	65.0 (#120)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)
Average weight of ovaries, mgm.	156.3	117.0	110.0	88.0	107.3	114.0	117.7	78.7	72.3	63.7	74.7	65.7	49.3
Activation	150%	95%	77%	29%	53%	78%	107%	78%	85%	95%	100%	91%	53%

hypophysectomized animal. Prolan has but a limited effect on the ovaries of the normal infantile rat. Hypophyseal hormone stimulates the infan-

TABLE 7

Effect on the immature rat ovary of prolan combined with hypophyseal preparations containing the gonad stimulating hormone but free of growth hormone

	DESIGNATION OF EXPERIMENT							
	377	373	372	375	389	388	369	399
Dose prolan, mgm...	13.6	0	13.6 (*337)	27.2	0	27.2 (*375)	54.5	54.5 (*369)
Dose A. H. H., mgm.	0	20.2	20.2 (*373)	0	65.0	65.0 (*389)	0	65.0 (*389)
Average weight of ovaries, mgm.....	32.3	25.7	32.7	32.7	13.7	43.3	52.0	43.7
Activation.....			None			Inconsiderable		None

TABLE 8

Effect on the ovary of the immature rat of combinations of prolan with hypophyseal preparations containing growth but free from the gonad stimulating hormone

	DESIGNATION OF EXPERIMENT								
	375	220	446	450	486	485	499	272	501
Dose prolan, mgm.	27.2	0	27.2 (*375)	27.2 (*375)	54.5	0	54.5 (*486)	0	54.5 (*486)
Dose A. H. H., mgm.....	0	112.5	45.0 (*220)	22.5 (*220)	0	24.0	24.0 (*485)	41.3	41.3 (*272)
Average weight of ovaries, mgm....	32.7	21.0	85.1	53.0	62.3	20.3	92.3	16.5	106*
Activation.....			160%	62%			48%		70%

* These ovaries were composed of 0–3 corpora and 7–20 large cysts. In this experiment the activation can not be explained on the basis of the additive effect of so-called "follicle stimulating" and "luteinizing" hormones.

tile genital system of the hypophysectomized dog and rat to full development. Hypophyseal hormone is not limited in its action on the infantile ovary as is prolan. Prolan can be made to have a maximal effect on the

ovary by combining it with an hypophyseal extract, which by itself is not potent in accelerating sexual maturity.

We offer the following explanation of these facts. The gonad-stimulating hormone is present in the hypophysis in an inactive state. The active substance is formed from this "prohormone" by an activator. This activator is found in the most concentrated form as prolan in the urine of pregnant women. It may also be present in the hypophysis itself, even in the infantile hypophysis. But if the activator is present in the hypophysis it is normally separated from the prohormone somewhat as glycogen is separated from glycogenase in the liver. In the normal infantile animal its effect is limited by the small amount of prohormone present. If we add more prohormone to the infantile animal's own store, we can stimulate by prolan the development of ovaries that exceed normal physiological limits. Our purest growth hormone preparations are activated in this way. Hence the prohormone may be the growth hormone; we have at any rate not been able to separate it from the growth hormone. On the other hand growth-free gonad-stimulating hormone from the hypophysis can not be further activated by prolan, doubtless because it is already completely activated or maximally potent.

These explanations seem to us to best fit the experimental findings though we realize that there are other possible interpretations than those offered. The simplest explanation would seem merely that there is an additive effect when prolan is combined with hypophyseal hormone. We believe that our experimental work has excluded this possibility. The ovary weights stimulated by the combination are larger than what would be expected on the basis of an additive effect. Further, prolan can be combined with potent sex hormone preparation from the hypophysis without showing the activation effect. Most crucial of all, prolan activates hypophyseal growth extracts which by themselves show no gonad-stimulating effect at any concentration.[10]

We believe we have excluded also a simple physical explanation of the activation phenomenon (tables 2 A, B, C). The absorption, excretion, or possible destruction of prolan might be delayed by the combination, and therefore the actual amount of hormone available to the affected organ might be greater. If the hypophyseal component is replaced by a non-specific substance of similar physical make-up—e.g., liver—the activation reaction does not occur. Similar activation fails if the hypophyseal component is destroyed by chemical reaction or mild heat which does not sensibly change its physical character.

Furthermore we do not feel that we have grounds for assuming that our findings can be explained on the basis of different proportions of so-

[10] Even at five times the concentration used in the activation experiment.

called "follicle-stimulating" and "luteinizing" hormones in the two components used in the activation reaction.[11] For example it might be assumed that prolan contains more follicle-stimulating hormone and that the hypophyseal extracts furnish an excess of the luteinizing principle and that on combination of the two products a summation of these effects occurs. This assumes that the luteinizing hormone cannot act on an ovary unless it is first stimulated by the follicle-stimulating hormone. Although the large ovaries in the activation reaction are usually composed chiefly of corpora, this is not always true. We have had cases of activation in which the heaviest ovaries produced in a given experiment were composed almost entirely of large follicles and follicular cysts.

We believe that a recent observation of Hill and Parkes on ovulation in decerebrate rabbits can be explained on the same basis as our experiments. Hill and Parkes found that the injection of the urine of pregnant women (prolan) was able only in slight measure to replace the action of the animal's own hypophysis in provoking ovulation in animals decerebrated immediately after copulation. It was effective in only a small per cent of cases "4 inferior results" resulting from 19 trials. The ovulation was delayed longer than usual and less ova were freed. Even this minimal effect was not possible when placental extracts were injected into these decerebrate rabbits. On the other hand the placenta of pregnant rabbits, which had been injected previously with the urine of pregnant women, provoked ovulation in the decerebrate animals. We assume that the development of the gonads of the immature rat is a comparable phenomenon to ovulation in rabbits, and that hypophyseal hormone was necessary for ovulation in the rabbits used in the Hill and Parkes experiments; in other words, that prolan was unable to function except in combination with hypophyseal hormone and that the few positive cases of ovulation reported by them were due to minimal amounts of hypophyseal hormone still circulating in the blood of the decerebrate animals, which, combined with the prolan injected or stored in the placenta of the urine-injected animal, was adequate to provoke ovulation.

CONCLUSIONS

1. Prolan does not easily repair the gonadal deficiencies of hypophysectomized animals (dog, rat).

2. There is always a definite limit to the weight of the ovary which can

[11] After the present communication was written, the paper by H. L. Fevold, F. L. Hisaw and S. L. Leonard on the gonad-stimulating and the luteinizing hormones of the hypophysis (This Journal, 1931, xcvii, 291) appeared, but too late for discussion here. In it activation effects are described as the result of combining two hypophyseal preparations which gave respectively the follicle-stimulating and luteinizing effects. We intend to discuss this matter fully in a later paper.

be stimulated by prolan in the immature rat within a definite time interval (100 hr.).

3. Hypophyseal hormones completely repair the gonadal deficiencies of hypophysectomized animals (dog, rat).

4. The hypophyseal gonad-stimulating hormone does not show the limited effect on the ovary of the immature rat found to be characteristic of prolan. The development of the ovary provoked by the hypophyseal hormone corresponds to the dose level.

5. Only very small amounts of prolan, measured in terms of dry weight, are required to give the minimal effect on the ovary. The amount of hypophyseal hormone needed to give a minimal effect is always much greater. This is in contrast to the fact that higher doses of hypophyseal hormone provoke much greater ovary weights than can be obtained with any amount of prolan.

6. In our earlier experiments the effect of prolan was increased to the maximal effect obtainable by injecting hypophyseal sex hormone simply by combining prolan with small amounts of crude hypophyseal preparations, containing both gonad-stimulating and growth hormones. The combination of prolan was made with doses of hypophyseal preparations which, given alone, were minimal or just subminimal in gonad-stimulating effect.

Later we added prolan to hypophyseal extracts (growth hormone) which, when administered alone, were devoid of any effect on the ovaries of immature animals and in this way also secured maximal effects.

7. This activation effect is a specific reaction. If the hypophyseal extract is destroyed by heating, the combination of heated hypophyseal hormone with prolan is no longer any more effective than prolan alone.

8. Low concentrations of pure hypophyseal sex hormone combined with prolan do not result in activation effects. On the other hand, sex-free growth hormone is typically activated by prolan.

RELATIVE INEFFECTIVENESS OF PROLAN IN HYPOPHYSECTOMIZED ANIMALS[1]

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Smith has shown that at least four clear cut effects follow complete removal of the anterior hypophysis in the young animal (rat), namely, undergrowth, sexual infantilism, and impairment of the thyroid and adrenal cortex. He has shown that all the deleterious effects from hypophysectomy can be obviated by a substitution therapy[2] in the form of frequent implants of rat anterior hypophyseal substance. Reichert[3] has similarly been able to repair the growth and gonadal deficiencies of completely hypophysectomized dogs by implants of rabbit hypophysis. We now possess potent extracts of the anterior hypophysis which appear to selectively affect the growth and sex mechanisms respectively. Smith was able to show that our extracts rich in the growth hormone could restore growth in hypophysectomized rats without, however, affecting any of the other three deficiencies. Perhaps the most complete accomplishment in this respect was the growth which Reichert[4] provoked by administering our aqueous alkaline extract intraperitoneally to hypophysectomized puppies; these animals exceeded in growth their normal controls.

The discovery by Aschheim that the urine of pregnant women is capable of provoking precocious sexual maturity in young rodents, a response which hitherto had been provoked only by implants of anterior hypophyseal tissue, led us to hope that this substance, Prolan, might mature the

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[2] Smith, P. E. The disabilities caused by hypophysectomy and their repair. Journ. Amer. Med. Assoc., 1927, lxxxviii, 158.

[3] Reichert, F. L. The result of replacement therapy in an hypophysectomized puppy: four months of treatment with daily pituitary heterotransplants. Endocrinol., 1928, xii, 451.

[4] Reichert, F. L. Effects of anterior pituitary extract upon an hypophysectomized puppy. Proc. Exper. Biol. and Med., 1929, xxvii, 204.

infantile sexual apparatus of hypophysectomized animals. Besides our desire to know whether Prolan could parallel the action of hypophyseal implants in the repair of gonadal deficiencies in such animals, we had an added interest in its injection, namely, to see whether repair of the thyroid and adrenal deficiencies paralleled gonadal repair.

A total hypophysectomy was done on three puppies when approximately six weeks of age following the method employed by Dandy and Reichert.[5] In all three cases within a few weeks it was evident from careful measurements of roentgenograms that growth stasis had resulted and that the operation was therefore complete. At various intervals after the operation, to be precise, at twelve, at two, and at six months respectively, high dosage Prolan administration was begun. In the case of the animal which had survived hypophysectomy for a year, daily high dosage for a month with Prolan produced no perceptible effects nor did a laparotomy at this juncture show that we had affected the infantile state of the ovary and uterus. The ovary had undergone an almost complete fibrosis and no follicles were discernible. Injections were resumed after the operation and continued for a period of 17 days. The dosage was higher than before but with similar non-effect. Autopsy at this juncture disclosed the other uterine horn as still threadlike and the ovary as small, hard, and white.

It seemed to us possible that the extensive regression and atrophy of the genital system might now have carried with it the lack of capacity to respond to a stimulus and that therefore such attempts should have been carried out within a short time after a complete hypophysectomy. For this reason a similar intensive Prolan dosage was given a puppy two months after a complete hypophysectomy, but in this younger case also no result was elicited.

In the meanwhile we had by simple procedures (re-solution and precipitation) purified and concentrated our Prolan so that it had approximately five times the strength of the material first employed. A puppy which had survived complete hypophysectomy for approximately six months and was in good health, was treated with the stronger Prolan. Fifty-three daily injections of about 30 cc. each were made without demonstrable effect on the sexual apparatus. An autopsy showed that both ovaries and uterus were infantile.

Inasmuch as the effect of Prolan in hastening sexual maturity has been ascertained in most cases by the use of small rodents (mice or rats) there existed the possibility, of course, of a better possible response in these forms. Therefore, a rat $3\frac{3}{4}$ months old and weighing 171 grams was hypophysectomized (Pencharz). Two months afterwards, the operation being demonstrably complete, 8 units of Prolan were given subcutaneously

[5] Dandy, W. E. and F. L. Reichert. Johns Hopkins Hosp. Bull., 1925, xxxvii, 1.

daily for six days without effect. The animal was allowed to rest; then dosage was continued for ten days longer. At the end of this period there was still no change in the vaginal lips and the vaginal smear and the animal was sacrificed. Ovaries and uterus were infantile. The detail of all four experiments will be found in the tabular protocols.

The lack of response of the genital system of these hypophysectomized animals to prolonged and repeated administration of Prolan of known potency indicates at any rate the incapacity of such animals to respond to a treatment which has pronounced effects on the ovary of the infantile normal animal.[6]

PROTOCOL

DATE	PROCEDURE	REMARKS
	Dog H-92, Female shepherd	
April 28, 1930	Total hypophysectomy with cautery at base of brain—Dr. Reichert	7 weeks old; weight 3.6 kilos (H-93, female litter mate control, 3.7 kilos)
April 28, 1930, to February 19, 1931	X-ray measurements of bones and body weight taken every two weeks	Gained 0.4 kilo, skull increased 0.6 cm., sum of tibia and femur increased 3.2 cm., never lost first denture (H-93 gained 12.7 kilos, skull increased 6.5 cm., femur and tibia increased 20.0 cm., second denture July 30, 1930)
December 9, 1930	Prolan injections begun	6 months post-operative 53 daily injections of approximately 30 cc. each (10 cc. subcutaneously, 20 cc. intraperitoneally) Potency of prolan, 1/8 cc. = 1 R. U.
February 20, 1931	Died	Pneumonia. Age 49.5 weeks No change in vulva suggestive of an effect on genital system. At autopsy, ovary and uterus proved to be infantile (H-93 came into heat Dec. 9, 1930)

[6] Since the above was written, a purification and concentration of Prolan has been effected by us; with the administration of 400 RU's daily to hypophysectomized rats, some instances of response of the genital system have been secured.

PROTOCOL—*Continued*

DATE	PROCEDURE	REMARKS
	Dog H-46, Female	
October 24, 1928	Total hypophysectomy —Dr. Reichert	Six weeks old
October 14, 1929	Prolan injections begun	One year post-operative→ Injections were made intraperitoneally daily. Total of 385 cc. given in course of 4 weeks = 14± cc. daily Prolan potency, 12 mgm. per cc. (1 mgm. = 1 R. U.)
November 11, 1929	Injections stopped	Because no external evidence of influence on genital tract appeared
	Laparotomy performed	One ovary and fragment of uterus removed. *Ovary*, small, hard and white. In section, *ovary* almost completely fibrous, no follicles discernible Weight of animal, 5 kilos
October 22, 1930	Prolan injections resumed and continued for 17 days	35–40 cc. prolan injected, intraperitoneally and subcutaneously, daily
November 8, 1930	Died	Death result of a fall Autopsy showed *uterus* thread-like; *ovary* small, hard, white. Typical of animals hypophysectomized for this period of time
	Dog H-80, Female	
October 21, 1929	Total hypophysectomy —Dr. Reichert	Six weeks old; weight 2.6 kilos. (H-79, female litter mate control, 23 kilos)
December 21, 1929	Prolan injections begun	Two months post-operative 15 cc. prolan (potency as for H-46) injected intraperitoneally, daily
January 17, 1930	Prolan injections	No external changes in genitalia; vulva infantile Weight of animal, 3 kilos (H-79, 9.3 kilos) Animal not sacrificed

PROTOCOL—*Concluded*

DATE	PROCEDURE	REMARKS
	Rat W-30, Female	
November 4, 1930	Total hypophysectomy —Mr. Pencharz	Age, 3¾ months; Weight, 171 grams
January 15, 1931	Operation to obtain control tissues	Left ovary, part of left uterine horn, one adrenal, and one thyroid removed. *Ovary*, infantile
January 19, 1931	Prolan injections begun	1 cc. daily, subcutaneously for 6 days Prolan potency: 1/8 cc. = 1 R. U.
January 25, 1931	Prolan injections stopped	No change in vaginal lips or in vaginal smear during period of injection or subsequent to it
February 16, 1931	Prolan injections resumed	1 cc. daily, subcutaneously for 10 days
February 26, 1931	Injections stopped	No change in vaginal smear nor in lips of the vagina Weight during injection period, 160 grams
May 11, 1931	Died	Weight, 148 grams. Ovary, infantile

RESPONSE OF EXPLANTED CARDIAC MUSCLE TO THYROXINE

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It seems to be well established from the work of Lewis and McEachern (1931), Priestley, J. Markowitz, and Mann (1931), and Yater (1931) that perfused hearts of thyroxinized rabbits pulsate at a much faster rate than the hearts of non-thyroxinized rabbits. Mann, J. Markowitz and Priestley (1931) found that the heart of a puppy that had been transplanted by vascular anastomosis into the neck of a larger dog beat much faster after thyroxinization of the host. These experiments indicated that the tachycardia of patients with hyperthyroidism is not due to stimulation of the accelerator nerves of the heart, as had been supposed, but to a direct effect on the heart. Yater (1931) performed experiments with perfused hearts of rabbits which showed that the action of thyroxine is on all parts of the heart, auricles and ventricles, and that the increase in rate is not dependent on the presence of the sino-auricular node. It remained only to be determined whether thyroxine acts on the muscle fibers directly or through the mediation of nerve ganglia or nerve endings. The present report deals with experiments performed to procure this information.

Since the surgical excision of the intracardiac ganglia is impractical, and even if it were practical there would still remain the so-called intermediary receptive substance of Langley, probably in a sensitized form, the only recourse available was the use of fragments of embryonic heart excised before the appearance of functioning nerve elements and kept alive by the modern methods of tissue culture. The most convenient preparation of this type is the explanted heart of the chick embryo kept alive in a suitable culture medium. Such fragments pulsate for many days, during which time there is a vigorous proliferation of fibroblasts in the culture medium and some proliferation of muscle cells. Ultimately, the latter die and only fibroblasts remain alive; as a consequence, the pulsations gradually become feebler and stop. In the interim the pulsating fragment of cells of the heart muscle is available for various physiologic experiments.

It was reported in a previous paper (Markowitz, 1931) that such fragments removed from two-day-old embryos were usually devoid of functioning nerve tissue, as judged by the response to epinephrine and acetylcholine.

Since this observation is consistent with the anatomic data of Szantroch (1930) that ganglia do not make their appearance until after the third day in hearts of chick embryos, it may be considered a safe premise that if thyroxine exerts its usual accelerating effect on the pulsations of a fragment it has accomplished this by direction action on the muscle cells. We have found that thyroxine accelerates the pulsations of cultures of cardiac fragments removed even from two-day-old chick embryos. It was possible to show quite clearly that the rate of pulsation of such fragments increased markedly following incubation with thyroxine, when, at the end of the experiment, they were beating vigorously but were utterly irresponsive to epinephrine and acetylcholine. We submit these experiments, therefore, as evidence that thyroxine accelerates the heart rate of intact animals by a direct effect on muscle cells, without the intervention of any nerve elements, or the so-called intermediary receptive substance.

During the research it happened that thyroxine was also added to cultures removed from older chick embryos (up to nine days). These are known to contain ganglia, and the existence of functioning nerve elements was readily demonstrable by the typical effects of epinephrine and acetylcholine on the rate of pulsation. The addition of thyroxine quickly accelerated the rate of such explants; the addition of epinephrine caused further acceleration, whereas acetylcholine stopped the pulsations for at least thirty seconds. In general, however, such preparations were no more sensitive to thyroxine than were nerve-free explants.

METHODS OF EXPERIMENTATION. It is hardly necessary to state that scrupulous asepsis was maintained throughout. Cultures were prepared from the hearts of chick embryos after the eggs had been incubated forty-eight hours or longer. To excise the hearts of very young embryos it was found necessary to use a dissecting microscope and very sharp instruments. The hearts were removed and cut into fragments about 1 sq. mm. in size. These fragments were explanted into Carrel flasks, type D, containing 0.3 cc. of heparinized chick plasma and 1.0 cc. of embryo extract.[1] Fifteen minutes after the medium with the embedded fragments had clotted, 1.0 cc. of embryo extract was placed on the surface. The cultures were incubated at 39°C. for four to twenty-four hours, during which time the rates of pulsation had become stabilized and could be counted prior to the addition of thyroxine. Usually fragments of each heart were placed in at least two flasks and one flask was kept as a control. Most fragments, soon after explantation, showed regular vigorous contractions which continued for days with only slight change in rate. Although each fragment varied

[1] Embryo extract was prepared (somewhat after the method of King, 1930) by placing an eight or nine-day-old chick embryo in 9.0 cc. of Tyrode's solution and shaking rapidly until the embryo was broken up. It was then centrifuged for ten minutes and the opalescent supernatant fluid was used undiluted.

little in rate of pulsation there were great variations in the rates of different fragments. Various methods were used to get a physiologically active solution of thyroxine that would be of uniform strength, simple to prepare aseptically, and of a pH that would not upset the reaction of the tissue culture itself. The method finally adopted was to weigh out thyroxine accurately in a small pyrex tube and add sterile distilled water in the proportion of 1.0 cc. of water to 0.02 mgm. of thyroxine. The tube was corked tightly and sterilized at 15 pounds pressure for fifteen minutes. When needed, it was shaken thoroughly and 0.1 cc. of this suspension was added to the culture. This culture, be it repeated, had 1.0 cc. of fluid embryo extract in addition to the solid matrix. Calculating that all the thyroxine went in solution the final dilution was 1:50,000, admittedly a very high concentration.

To count the pulsations the cultures were removed from the incubator from time to time and placed on the stage of the microscope which was enclosed in a hot-box kept at 39°C. In a few minutes after stabilization of the temperature the pulsations became regular and counts were made. After the addition of thyroxine, counts were again made at various intervals, and when the rate had markedly increased, 0.1 cc. of weak solutions of epinephrine and of acetylcholine were added to the fluid bathing the pulsating fragments. After each addition of epinephrine or acetylcholine this fluid was pipetted off and replaced by the same amount of warm Tyrode's solution. This was left on for a moment, then removed, to be again replaced by warm Tyrode's solution. As a matter of interest it may be stated that some of these cultures were subjected to further incubation, in which case fresh embryo extract was added.

Results. A few typical experiments will best illustrate our results. Fragments from a two-day-old embryo heart were explanted in the usual manner and incubated for eighteen hours. At this time the rate of one fragment was 120 for each minute and the other fragment in a separate flask had a rate of 85. To the latter flask thyroxine was added. Six hours later the rates were 92 and 70, respectively. In twelve hours the rates were 100 and 185, respectively. When acetylcholine and later epinephrine were added to the fragment that had responded to thyroxine, the rate remained constant at 185. By the next day the thyroxinized culture was not pulsating. Irrigation with Tyrode's solution caused it to resume beating at a rate of 218, which six hours later had increased to 240. By the next day the pulsations had stopped and no attempt was made to revive them. At this time the control was beating regularly at 85 although prior to this it had remained constant at a rate of 100 for each minute.

In a similar experiment on a fragment of the heart of a two-day-old embryo the rate was 150. Six hours after thyroxinization the pulsations were slightly irregular both in rate and vigor, but after twelve hours the

rate was regular at 185 for each minute. In twenty-four hours it had increased to 226. At this time it did not respond to epinephrine or acetylcholine; six hours later it was quiescent. The control fragment remained pulsating at about 100 and responded to the addition of epinephrine by a temporary increase in rate to 171; it did not respond to acetylcholine.

A four-day-old fragment responded to thyroxine by an increase in rate from 171 to 240. The addition of epinephrine caused a further increase to 300. A similar fragment with a rate of 120 responded to thyroxinization by an increase to 160. When epinephrine was added the rate increased to 240. Acetycholine caused it to stop pulsating for one minute and then it regained the rate of 150.

COMMENT. These experiments bring to light the inherently probable observation that thyroxine accelerates the pulsation of cells of the heart muscle in a manner that cannot be attributed to any intervening nerve tissue. It is of interest to mention certain other effects of thyroxine on cultures of the cells of heart muscle apart from this effect on the rate of pulsation. Although the massive injection of thyroxine given to a dog is not followed by pronounced tachycardia for twenty-four hours, the addition of thyroxine to cultures of cells of pulsating muscle is often followed by a well-marked effect in twelve hours. In the experiment mentioned, the rate of a nerve-free culture increased from 85 to 185 a minute in twelve hours. Twenty-four hours after the addition of thyroxine it was quiescent until irrigated with a little Tyrode's solution to wash away the products of fatigue, following which it promptly began to beat at a rate of 218. Six hours later the rate was 240 in spite of the addition of acetycholine. By the next day the pulsations had stopped and no attempt was made to revive them. Sometimes it was possible to observe the culture fibrillating. The addition of thyroxine had evidently caused a progressive rise in the rate, resulting in fibrillation and finally in paralysis. It was frequently noted (these experiments were discarded) that following thyroxinization, periods of irregularity might interrupt the uniform rapid rate of the explant. Thus the culture might beat rapidly for five seconds and resume its former rate, or it might beat rapidly for a few seconds, then stop for as long as forty seconds and resume some type of pulsation with or without pauses at a slower or faster rate. Another observation that is probably significant is that cultures which had been subjected to these large amounts of thyroxine did not pulsate for as many days as did the controls. We are inclined to attribute this to the exhaustion of the muscle cells and the accumulation of fatigue products. These observations have, of course, a bearing on analogous states of the heart of patients with hyperthyroidism.

This is not the place to consider the physiologic basis for the clinical syndrome that has come to be recognized as hyperthyroidism, except to indicate that such salient features as exophthalmos, tremor, and tachy-

cardia have, for decades, been attributed to sympathetic over-stimulation. It may be taken as definitely proved that this conception is erroneous for tachycardia. The newer knowledge of the sympathetic innervation of skeletal muscle renders it improbable that the tremor is sympathetic in origin. Leaving other considerations of the possible mechanism aside, it is unlikely that the exophthalmos is of sympathetic origin, unless the site of stimulation is very specific. It is true that stimulating the cervical sympathetic nerves of animals may cause exophthalmos, but it also produces dilatation of the pupil and unilateral constriction of the blood vessels. It is illogical to attribute the exophthalmos to general sympathetic stimulation in the absence of these other accompaniments. If there were such a clinical syndrome as violent sympathetic over-stimulation its picture would be that of overdosage of epinephrine, a picture with which every clinician has become acquainted. Except for the tachycardia, this picture had nothing in common with the symptoms of hyperthyroidism.

SUMMARY

A study was made of the action of thyroxine on cultures of pulsating fragments of heart muscle removed from two-day-old chick embryos, before the appearance of nerve elements. Thyroxine exerted its typical effect on the frequency of pulsation and contractility of such fragments, bringing about a progressively greater increase in rate ending in some cases in fibrillation and paralysis. These results constitute evidence that in clinical hyperthyroidism the tachycardia is due to the action of thyroxine directly on the myocardium and not on intervening nerve elements.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XXIII. The Rôle of the Liver in the Destruction or Inactivation of Nicotine

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It has been shown by Priestley, Markowitz and Mann (1930) that the ability of the liver to destroy strychnine is many times greater than that of an equal mass of skeletal muscle. The question naturally arises whether the liver possesses this marked ability to inactivate other highly toxic alkaloids. We shall report here the results of our investigation of the part played by hepatic tissue in the destruction or inactivation of nicotine. This alkaloid was chosen for the reason that it can be estimated biologically in small quantities. The method for doing this depends on the familiar property of nicotine to raise blood pressure. If 0.5 mgm. of nicotine is injected intravenously it will raise markedly the blood pressure of a dog weighing 5 kgm. as will the injection of 0.1 cc. of 1:1,000 epinephrine. As in the case of epinephrine the blood pressure soon falls to the original level, and when the injections are not made more often than every fifteen minutes a remarkably uniform response can be obtained by repeated injection of the same amount over a period of two hours.

In our investigations the dog and the frog (Rana pipiens) were used. If the dog was used the methods employed were those described by Priestley, Markowitz and Mann. In brief, the nicotine present in the blood was assayed in terms of its vasopressor action after passage through a heart-lung, a heart-lung-liver, a heart-lung-limb circuit and also on its action on the normal and dehepatized dog. Sufficient nicotine was dissolved in a small quantity of Ringer's solution and added to the venous reservoir of the heart-lung circuit to make a concentration of about 0.1 mgm. for each cubic centimeter of blood. Appropriate samples of blood were removed at intervals and injected into an etherized dog weighing 5 kgm.; 5 cc. samples were used as a routine.

The effect of nicotine was observed on normal, splenectomized, par-

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tially dehepatized and totally dehepatized frogs following injection into the dorsal lymph sac of 0.1 mgm. for each 10 grams of body weight. The time required for complete recovery from the prostration produced by the injection was used as the criterion of the detoxifying ability of the frogs of the respective series. The frogs were used four days after operation. This precaution was taken to eliminate the frogs that were destined to succumb as a result of operation; Mann and his co-workers have shown that if totally dehepatized frogs live for four days they usually live several days longer.

Representative experiments. Two liters of blood were obtained from healthy donors and this was added to the venous reservoir of a heart-lung circuit. A dog weighing 10 kgm. was then etherized and its heart and lungs were connected in the usual manner with the heart-lung circuit. With maximal dispatch the liver was later placed in circuit in the manner described by Priestley and his co-workers. At this stage a dog weighing 5 kgm. was etherized and the blood pressure was taken through the carotid artery and recorded in the usual manner; 200 mgm. of nicotine were now added to the venous reservoir. The effect of the nicotine on the heart of the heart-lung circuit is interesting. After the addition of the nicotine it became markedly slow and irregular for five minutes. Then it was accelerated for about five minutes following which it behaved as it did before the introduction of nicotine. As a control the first sample of blood for assay was taken from the venous reservoir. Subsequent observations were made with samples of blood coming from the liver. Samples (5 cc.) were taken about fifteen minutes apart and the effect on the blood pressure recorded after intravenous injection into the dog that was used for the assay. The first sample gave the predictable result, namely, the effect produced by 0.5 mgm. of nicotine. Samples coming from the liver fifteen minutes after the addition of nicotine showed large diminution in the effect of nicotine. Successive samples showed less and less action. Seventy minutes after the introduction of nicotine the effect on blood pressure of a 5 cc. specimen of blood emerging from the liver was not appreciable. The injection at this point of 0.5 mgm. of nicotine into the etherized dog provoked the usual rise in blood pressure (fig. 1).

It is apparent that the liver possesses the ability to remove large quantities of nicotine from blood that has been perfused through it. Whether the nicotine is absorbed or destroyed was not determined. The fact that it disappeared completely within seventy minutes would suggest that it was destroyed by the liver.

It might be supposed that the nicotine undergoes the same alteration either in the heart or lungs or in the perfusing medium. To test this we performed an experiment on a heart-lung preparation without the liver

in circuit. The amount of nicotine in the blood did not show any significant change over a period of two hours, as was tested by the effect on the blood pressure of another dog.

To compare the ability of skeletal muscle to inactivate nicotine we prepared a heart-lung-limb circuit. The venous reservoir contained 1,200 cc. of blood. After ligating the aorta, a cannula was placed rapidly in the femoral artery and rapid amputation at the hip was performed. The limb was placed in the incubator and the femoral artery was connected with the heart-lung circuit proximal to the peripheral resistance. To the venous reservoir containing 1,200 cc. of blood was now added 120 mgm. of nicotine, otherwise the experiment was conducted as in the preceding case. As judged by the influence on blood pressure the rate of destruction of nicotine was definitely slower by the hind limb than by the liver.

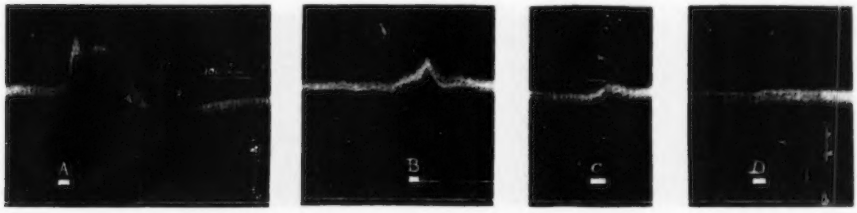

Fig. 1. The effect on the blood pressure of successive injections of 5 cc. of blood which contained approximately 0.1 mgm. of nicotine for each cubic centimeter at the beginning of the experiment. The response of the blood pressure to 5 cc. of blood removed from the venous reservoir previous to perfusion through the liver is shown at *A*. After the blood had been perfused through the liver for fifteen minutes the standard dose produced the response shown at *B*. The effect of the standard dose was weak when the perfusion had lasted forty-five minutes, *C*. At the end of seventy minutes there was no appreciable effect on the blood pressure, *D*.

The blood flow from the femoral vein was vigorous, amounting to about 150 cc. a minute. The blood issuing from the vein was venous in color. Fifteen minutes after the introduction of nicotine into the venous reservoir there was a sharp fall in the nicotine action to about half of its original value. Instead of diminishing progressively, however, it remained at this level in spite of the fact that the experiment was continued two hours. It would appear from these results that the nicotine diffuses and is perhaps absorbed by the muscular tissues, which may possibly account for the initial fall in the concentration. Much less nicotine disappeared subsequent to the initial fall than took place in the liver.

The effect of nicotine on the blood pressure of a dog was determined before and after hepatectomy. For this purpose a cannula was placed in the femoral artery. Following an injection of 1 mgm. of nicotine there

was a moderate pressor response (fig. 2). After the liver was removed the blood pressure was taken in the same manner. The response to a proportionate dose (0.95 mgm.) was much more pronounced than that before hepatectomy (fig. 3). The duration of the response before hepatectomy was about one minute, but after hepatectomy it was three minutes. This experiment also furnishes suggestive evidence that the liver plays a significant part in the inactivation of nicotine.

Further data were obtained by the study of normal, splenectomized, partially dehepatized and totally dehepatized frogs. The operative procedures were similar to those followed in previous investigations reported from this laboratory. It was found that an injection of 0.1 mgm. of nicotine for each 10 grams of body weight into the dorsal lymph sac of normal frogs caused complete prostration within one to two minutes.

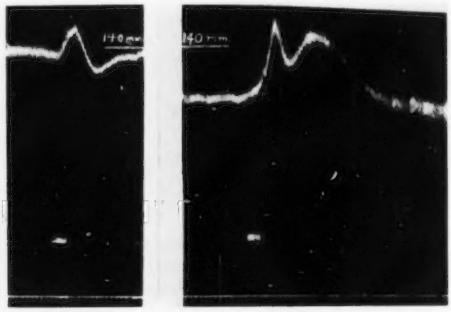

Fig. 2 Fig. 3

Fig. 2. The blood pressure following an injection of 1 mgm. of nicotine into a dog weighing 12 kgm. previous to hepatectomy.

Fig. 3. The effect of 0.95 mgm. of nicotine after hepatectomy.

The initial response was a convulsive seizure of short duration, after which the frogs remained immobile until recovery or death. The time required for complete recovery, which was considered to have occurred when the frogs were able to hop after mild stimulation and right themselves when placed on the back, ranged from fifty-two minutes to three hours. The majority, however, recovered within two hours. The foregoing dose of nicotine was not lethal in a single instance when injected into thirty-six normal frogs.

The effect of the nicotine on totally dephepatized frogs was decidedly more toxic. In each instance the usual primary convulsion and prostration followed injection, but in many cases a second convulsion occurred from five to ten minutes after the first, following which the frog appeared dead, but usually the heart continued to pulsate slowly for varying

periods before stopping completely. Of fifteen totally dehepatized frogs ten died and five recovered. With one exception the time required for recovery far exceeded that for the normal, splenectomized and partially dehepatized frogs (fig. 4).

COMMENT. It is, of course, difficult to make the experiments on the heart-lung-limb the exact equivalent of those performed on the liver. However, the results obtained on a dog before and after hepatectomy were comparable in that the effect of the nicotine was more pronounced and of longer duration after removal of the liver. Furthermore, partially dehepatized and totally dehepatized frogs were more profoundly affected by a given dose of nicotine than the normal or splenectomized animal.

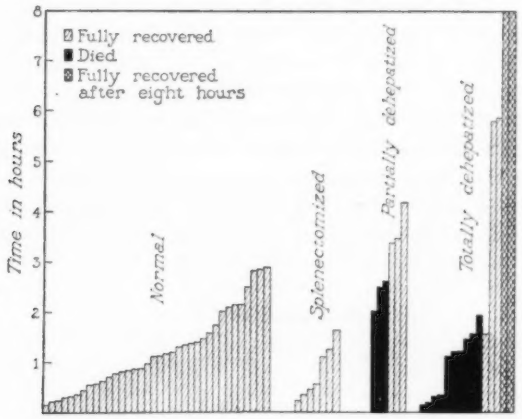

Fig. 4. The time of recovery or death of each of the normal, splenectomized, partially dehepatized and totally dehepatized frogs, following an injection into the dorsal lymph sac of 0.1 mgm. of nicotine for each 10 grams of body weight.

The fact that a high percentage of the totally dehepatized frogs succumbed, and that normal frogs survived following the same dose of nicotine indicates the importance of the liver in rendering this drug nontoxic. The long period required for complete recovery of dehepatized frogs indicates that the process of inactivating nicotine operates in the absence of the liver but proceeds at a much slower rate. From whatever angle the results are considered, the conclusion seems unavoidable that the liver is more active than other organs in removing nicotine from the circulation.

A number of suggestions may be offered as to the cause of this function of the liver to inactivate nicotine: 1, if the ability to destroy nicotine is a function of living tissue it is possible that an active tissue like the liver

would possess more of this ability; 2, since the liver structurally is like a sponge, the contact of the hepatic cells with the vascular circulation is an intimate one; the possibility for surface phenomena, that is, absorption, condensation, and dehydrogenation becomes facilitated, and 3, it is probable at any given moment that there is present in the liver a number of highly reactive chemicals which may easily combine with nicotine as it courses through this organ. Which of these methods is responsible for the marked ability of the liver to remove toxic substances from the circulation is a subject for further research.

SUMMARY

The ability of the perfused liver to destroy nicotine was studied by means of heart-lung preparations. The nicotine was estimated by its effect on the blood pressure of a small dog. Control experiments were performed by perfusing the hind limb in a similar fashion. It was found that the ability of the liver to destroy nicotine was definitely greater than that of an equivalent mass of hind limb. The action of a comparable dose of nicotine on the blood pressure of a dog after hepatectomy was much more pronounced than before hepatectomy. Following equal doses of nicotine, normal and splenectomized frogs recovered much more rapidly than partially and completely dehepatized frogs.

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DOES PHYSIOLOGICAL EXCITATION INFLUENCE PERMEABILITY IN STRIATED MUSCLE?

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In his book on permeability (1929) the senior author gave a summary of the relationship between excitability and permeability. On the basis of the work of Gildemeister, Ebbecke, Embden and others he concluded that increase in permeability ordinarily accompanies the transition from the resting to the excited state. It was assumed that we were dealing here with a general law of physiology holding for animal cells as well as for plant cells.

Some recent investigations seem to show that this assumption is not universally true. A great number of investigations, especially those of Embden and his co-workers have been performed on excised muscles with direct application of the electrical current. Although the effects of electrical stimulation were quite reversible and even subminimal stimuli evoked an increase in the output of phosphoric acid, it seems doubtful that the results obtained under these conditions can be applied to physiological excitation since Winterstein has shown that the local effects of electrical stimulation on the nerve are distinctly different from those of the propagated impulse. These experiments make it probable that a sharp distinction is to be made between electrical stimulation and physiological excitation. Since a propagated impulse set up by an electrical stimulation of the nerve corresponds completely to the physiological stimulation of the muscle, it seems necessary to investigate the question as to the permeability changes in muscle during excitation by means of indirect stimulation. Observations of Ernst and Csúcs (1929) and Mond and Netter (1930) had under such conditions negative results. These authors were unable to detect an increase in ion intake of perfused muscle when the muscles had been stimulated indirectly, and they concluded from their experiments that excitation is not accompanied by increase in permeability.

The question arises whether the negative results of Ernst and Mond might not simply be due to their methods. It is rather probable that permeability changes occurring in muscles under the influence of the physicochemical environment lead to significant changes in the intake of the effective substances. That holds, for instance, for Gellhorn's

studies on direct and indirect SCN contracture (1931). Therefore the question was investigated as to the permeability effects of indirect stimulation of the muscle by means of its functional reaction. Since the previous work of Gellhorn (1928) on KCl contracture seems to indicate that its strength, other conditions being equal, depends on the permeability of the muscle to KCl, the influence of indirect stimulation upon that contracture was studied.

METHOD. The experiments were performed on nerve muscle preparations (m. gastrocnemius) of R. esculenta from October 1930 to March 1931. The preparation was set up in aerated Ringer solution and connected with a light isotonic lever, which gave a magnification of eighteen-fold. The nerve was in a moist chamber and rested on platinum electrodes. Either faradic currents obtained from an induction coil with one dry cell in the primary current or condenser discharges were used for stimulation.

TABLE 1

*Height of contracture**

EXPERIMENT VI B	EXPERIMENT X B
mm.	*mm.*
1. 14	1. 11
2. *23***	2. 12
3. 18	3. *21*
4. *21½*	4. 14
5. 17	
6. *20*	

* "Height of contracture" is the distance the recording pointer rose above the base line, and represents the shortening of the muscle magnified eighteen-fold.

** The values in italics indicate the height of contracture during stimulation, the others being the control without stimulation.

The muscle was immersed periodically in a mixture of isotonic KCl (0.85 per cent) with Ringer's solution. The proportions were usually of the order of three parts KCl solution to five parts Ringer's solution, being varied for different preparations to produce a contracture of convenient magnitude. The proportion used in any one experiment was, of course, always the same. After each immersion the muscle was carefully washed in Ringer and then returned to aerated Ringer, where it was allowed to recover for ten minutes before another immersion. For control the muscle was merely immersed in the contracture producing solution. It was then immersed in this solution while being stimulated by break shocks of the inductorium (the secondary coil was set so that the make shocks were ineffective), or by discharges of a condenser (Scheminsky's apparatus). The frequency of stimulation used was between 60 and 120 per minute—frequencies which, for the durations used, caused no fatigue

contracture. The KCl contractures were recorded on a slowly revolving drum and allowed to reach a maximum.

Not all experiments showed an increased effectiveness of the potassium ions during stimulation. It was frequently difficult to obtain small contractions of the muscle, and large excursions of the lever were liable to destroy the effect, because the inertia of the falling lever during relaxation tended to stretch the muscle and counteract the effect of the potassium, since the tension set up by KCl is rather low. When conditions could be made optimal, however, the muscle revealed an increased susceptibility to potassium ions during stimulation. This is shown in table 1.

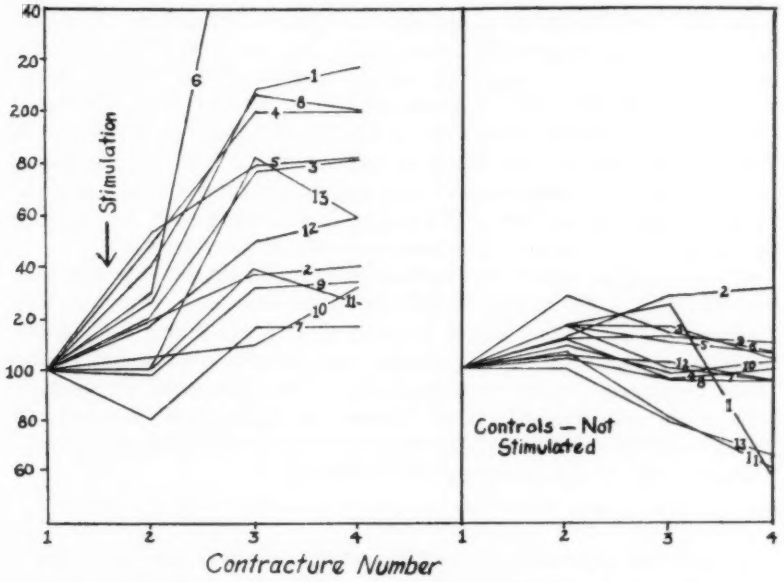

Fig. 1. The influence of indirect stimulation on the height of KCl contracture. Ordinate: Height of contracture expressed in percentages of the first one. Values (3) and (4) for curve 6 are 324 per cent and 354 per cent respectively.

In these experiments the two types of treatment of the muscle were alternated; i.e., first an immersion without stimulation, then with stimulation, then without, etc., the immersions without stimulation being for controls. The experiments show an increase in the height of the KCl contracture during stimulation.

A second type of experiment consisted in stimulating a nerve-muscle preparation *before* its immersion in the potassium solution and determining the influence on susceptibility to potassium ions. The procedure was as

follows: Two nerve-muscle preparations from one frog were used. First, each was immersed in the contracture producing solution and the contracture recorded. Then one muscle was stimulated with break shocks of the inductorium (which was set at a point where the make shocks were ineffective) at a frequency of 90 per minute. This stimulation was continued for five minutes, then stopped and a potassium contracture recorded, both in the stimulated and in the control muscle. It is notable that the threshold to electrical stimulation was not changed. Further potassium contractures were recorded in both muscles at intervals of twenty minutes thereafter. In very nearly all the preparations used, stimulation in this manner produced an increased susceptibility to potassium ions, as revealed by an increased height of contracture. In a few preparations this heightened susceptibility was not evident immediately after stimulation, but appeared twenty minutes later when a second contracture was recorded. Thirteen of these experiments were performed. The results are shown graphically in figure 1 in which the height of the first KCl contracture was taken as 100 per cent. The control value, obtained before stimulation, and subsequent contractures were expressed as percentages of the first. The graph shows very clearly the increase in susceptibility to KCl in the previously stimulated muscle.

The experiments show that indirect stimulation leads to an increased susceptibility of the muscle to KCl as is apparent in a study of KCl contracture. Since the muscle is permeable to K (compare Gellhorn, 1929), the conclusion seems justified that indirect stimulation of the muscle causes an increase in permeability. The experiments are in good agreement with observations of Thörner on degenerated muscles. In those, fibrillary twitches could be increased or provoked when the muscles perfused with KCl were stimulated indirectly.

SUMMARY

1. With two different experimental procedures it is shown that indirect stimulation of the muscle leads to an increased susceptibility to KCl contracture. This effect is obtained although the stimulation does not increase the threshold.

2. From these observations the conclusion is drawn that indirect stimulation leads to an increase in permeability of muscle.

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THE DIGESTION AND ABSORPTION OF RAW STARCH IN DOGS

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Several studies have been made concerning the digestion of raw starch. Langworthy and Deuel (1920) found the coefficient of digestibility in man to be nearly 100. More recently Roseboom and Patton (1928) found that dogs were able to digest raw corn starch in large amounts. Their method, similar to that of Langworthy and Deuel, consisted in the chemical examination of feces from dogs which had been fed large amounts of this carbohydrate. The feces were subjected to the iodine test for starch and were found to be uniformly negative. The material was then hydrolysed with dilute acid and tested for glucose. No glucose was found. They fed up to 75 grams raw cornstarch to dogs weighing about 15 kilograms, and concluded that dogs digested this quantity.

These authors also studied the amylolytic activity of extracts and secretions of dogs' salivary and pancreatic glands and believed that the pancreatic amylase was comparable in activity to that of omnivorous animals. Rosenthal (1929) fed raw starch to rabbits and other mammals and followed the curve of blood-sugar. He concluded that starch was digested and absorbed so slowly that the blood-sugar was not appreciably elevated. He fed important amounts of acid with the starch in order to delay its passage through the gut. More recently still Childrey, Alvarez and Mann (1930) have reported studies on digestion in dogs after colectomy. Their work suggested that there is little or no digestion of raw cornstarch in dogs and this interpretation has been made from their report. It is to be noted that these animals had a shortened digestive tube, that their findings were not based on chemical analyses, and that they were not definitely seeking for a proof of the digestibility of starch. They were searching for a "low residue" diet and were not chiefly interested in the chemical nature of the residue.

Because of this apparent disagreement we are led to report this experiment in which phlorizinized dogs were the subjects. We chose the phlorizin method as one which should definitely establish the facts of digestion and absorption of starch. This method on dogs has given good results in the hands of numerous investigators: Lusk (1912), Csonka (1916), and Olmstead (1920).

Two female dogs weighing 15.5 and 17 kilograms respectively were given daily injections of phlorizin in oil, 1.2 grams. They were also given several daily hypodermic injections of 0.2 cc. 1 to 1000 adrenalin hydrochloride. The urine was collected in 24 hour periods. The dogs were fed 250 grams of raw, ground, lean beef daily. The total nitrogen was determined by the Kjeldahl-Gunning method and the glucose by the method of Hawkins and Van Slyke (1929). The D/N ratios were calculated daily. When the D/N ratio approached 3.65, after a few preliminary days, the raw cornstarch was added to the ration for one day and determinations continued until the D/N ratio again approached 3.65. The results are indicated in the table. The data on the other dog were closely similar. The glucose derived from protein is obtained by using the factor $N \times 3.65$. The remainder of the glucose or "extra" glucose is derived from the starch. The result shows a nearly quantitative digestion of the raw starch. Commercial cornstarch contains about 9 per cent of water.

Starch digestion, dog 1. Female, 15.5 kilos

Daily injection of 1.2 grams phloridzin in olive oil

DATE (1930)	FOOD	URINE						
		Amount in 24 hours	Nitrogen per cubic centimeter	Nitrogen total	Glucose from protein	Glucose "Extra"	Glucose total	D/N ratio
		cc.	*grams*	*grams*	*grams*	*grams*	*grams*	
April 16	240 grams lean meat							
April 17	240 grams lean meat	1,010	0.0134	13.53			78.70	5.81
April 18	240 grams lean meat	1,000	0.0155	15.50			65.00	4.19
April 19	240 grams lean meat	1,100	0.0144	15.84			58.60	3.7
April 20	240 grams lean meat + 50 grams raw starch	1,500	0.0094	14.10	51.47	38.53	90.00	6.38
April 21	240 grams lean meat	1,500	0.0087	13.05	47.63	7.12	54.75	4.19
April 22	240 grams lean meat	2,000	0.0081	16.20			59.00	3.64

SUMMARY

By the use of the phlorizin method it appears that dogs digest large quantities of raw cornstarch.

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THE EFFECT OF COMPLETE SUPRARENALECTOMY ON THE OESTRUAL CYCLE OF THE WHITE RAT WITH REFERENCE TO SUPRARENAL-PITUITARY RELATIONSHIP[1]

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The experimental study of suprarenalectomy in the white rat and its effect upon the oestrual cycle has interested many investigators in the last few years. Kitagawa, in 1927, stated that following double suprarenalectomy the oestrual cycle in young and adult white rats was lengthened, became irregular or was completely inhibited. Wyman reported similar results adding that the inhibition or lengthening of the cycle was dependent upon the degree of suprarenal insufficiency. No attempt was made by either author to study the factor or factors involved in the effect on the cycle. Contrary to these findings are those of Lewis, del Castillo and Schiffer and Nice who concluded from their experiments that the oestrual cycle remains undisturbed after both suprarenals are removed. These contradictory reports have led the author to re-investigate the problem in an attempt to determine the exact results of complete suprarenalectomy and the factors concerned.

I. EFFECTS OF COMPLETE SUPRARENALECTOMY UPON THE OESTRUAL CYCLE OF WHITE RATS. The vaginal contents of 121 adult female white rats (4 to 15 months old) obtained from three different colonies, were examined after the method of Long and Evans and only those with at least three normal successive cycles (4 to 6 days) were used for experimental work. Complete suprarenalectomy was performed in two stages with an interval of 3 to 4 days between the removal of the first and second gland. The operative technique was uniformly a dorso-lateral lumbar approach requiring 8 to 10 minutes from the beginning of ether anesthesia. The gland, its pedicle and all surrounding connective tissue and fat were removed and the peritoneal wall and the skin sewed up. All controls, 32 in number, were unilaterally adrenalectomized on the right or left side. In addition, a special control series of 11 female rats was subjected to the following treatment: splenectomy, unilateral suprarenalectomy, thyroidectomy, ovariectomy and salpingectomy, allowing some

[1] This problem was aided in part by grants from the National Research Council administered by Dr. F. L. Hisaw.

twenty-two days for the entire series of operations. This was done to determine more completely the effect of surgical trauma on the oestrual cycle and the success, in general, of the operator's technique. Daily body

TABLE 1

Effect of total suprarenalectomy on the oestrual cycle of adult white female rats

All were virgin females, 4–6 months old, except where noted.

EFFECTS ON OESTRUAL CYCLE AFTER REMOVAL OF 2ND SUPRARENAL	NUMBER OF RATS	OESTRUAL CYCLE		NUMBER DIED	PERIOD OF SURVIVAL	REMARKS
		Pre-operative	Post-operative			
		days	*days*		*days*	
1. Complete inhibition of oestrum	22	4.5–6 Av. 5.3	No cycles	22	8–17 Av. 11.1	5 were multiparae over 1 year old
2. One oestrual cycle followed by complete inhibition	38	3.5–5 Av. 4.4	6–12 Av. 7.7	36	9–19 Av. 16.6	12 were 8–12 months old. No cortical tissue found in two surviving rats
3. Two oestrual cycles followed by complete inhibition	14	4–5 Av. 4.7	7–11 Av. 8.0	13	14–26 Av. 19.3	10 virgin females 8–12 months old. No cortical tissue found in surviving rat
4. Several irregular or prolonged cycles	32	4–6 Av. 4.9	7–14 Av. 8.4	17	14–27 Av. 21.1	Accessory cortical tissue found in 8 of the 15 surviving rats
5. No effect	15	4–5 Av. 4.3	4–6 Av. 4.7	9	7–9 Av. 8.0	Accessory cortical tissue found in 5 rats
Totals and averages.....	121	4.7	8.0	97	15.2	

Death from total suprarenalectomy = 80.1%—oestrual disturbance = 87.6%

weights, vaginal smears and rectal temperatures were recorded of these 11 females for at least three weeks after the last operation. The remaining suprarenal was then removed from three of these rats. Daily body

weights were recorded also of most of the suprarenalectomized females used in this study.

Table 1 summarizes the results of all completely suprarenalectomized females with the exception of the few which died within 5 days after the removal of the second gland. Data on such rats were not included since the death during this period was regarded as an immediate post-operative effect. All recorded deaths were of rats showing the typical signs of suprarenal insufficiency in laboratory animals.

In 87.6 per cent of the cases, complete suprarenalectomy causes definite but varied effects upon the oestrual cycle. These have been grouped in order of severity in column 1, table 1. Almost all rats showing no more than two oestrual periods after the removal of the second gland died within a period ranging from 11.1 to 19.3 days. This is especially true of those females showing complete oestrual inhibition. Such rats also have the shortest longevity. In rats showing oestrual cycles, the period of survival is seemingly prolonged by the interval of a normal oestrum although the occurring cycle is much prolonged (average 8 days). Further, the vaginal smears of these females were usually atypical. When females showed several prolonged or irregular cycles, the period of longevity was longest and 43.8 per cent of these survived. Accessory cortical tissue was found in only 8 out of the 15 surviving rats of this group. These females survived and showed irregular cycles for at least six weeks.

In 12.4 per cent of the operated females, no appreciable effect on the oestrual cycle was noticed although strikingly 9 of this group died from glandular insufficiency. It is to be noted that the survival period of these females was exceedingly short. Of the surviving 6 rats, only 3 possessed accessory cortical tissue. Of all operated animals, 80.1 per cent died of suprarenal insufficiency, the period of survival ranging from 7 to 27 days (average 15.2 days).

Histological studies of the ovaries of females showing complete oestrual inhibition immediately following total suprarenalectomy, revealed solid masses of corpora lutea which apparently had persisted for a long time. No mature or large follicles were present, and only a few small undeveloped ova were seen. The germinal epithelium of the ovary was broken up and this and the region between the corpora lutea were often replaced by areas of degeneration. Macroscopically, these ovaries were about $\frac{1}{3}$ to $\frac{1}{2}$ the size of the control gonads.

The ovaries of females showing suprarenal effect 4 (see table 1) were, in most histological respects, similar to the controls. The microscopic size was approximately normal and cross-sections revealed only a few more larger corpora lutea. Mature follicles were just as numerous. In females showing effect 3, the gonads are apparently in an intermediate stage. The size varied slightly from $\frac{3}{4}$ to full size. The corpora lutea

were large and numerous and occasionally a mature follicle would be seen. The number of undeveloped ova was variable.

Table 2 shows the results of control female rats which have been unilaterally suprarenalectomized and 11 others which, in addition, had several more organs removed from time to time. The operations were performed at various stages of the oestrual cycle. It can readily be seen that simple operative procedures, even if performed in short successive intervals, do not disturb the oestrual sequence at all. The daily rectal

TABLE 2

Effect of unilateral suprarenalectomy and other operative procedures on the oestrual cycle of adult control white rats

OPERATION PERFORMED	NUMBER USED	OESTRUAL CYCLE		NUMBER DIED
		Pre-operative	Post-operative	
		days	*days*	
Unilateral suprarenalectomy	32	3.5–6 Av. 5.2	4.5–6 Av. 5.3	0
Successive operations	11	4–5 Av. 4.8		
Unilateral suprarenalectomy			4–6 Av. 4.7	0
Splenectomy			5–6 Av. 5.5	0
Unilateral ovariectomy and salpingectomy			5–6 Av. 5.3	0
Unilateral thyroidectomy			5–6 Av. 5.6	0
Totals and averages.....	43	5.0	5.28	0

temperatures remained normal at all times, the body weights fell slightly (3–9 grams) but rose again to normal on the second day after each operation. All control rats showed normal oestrual periods and increases in body weight for at least 25 days after the last operative procedure. The surgical trauma of these operations is thus a negligible factor in oestrual disturbance. After the removal of the second suprarenal gland in three of the 11 "special control rats," death from typical suprarenal insufficiency followed in 14, 15 and 22 days. The oestrual cycle was inhibited in all three animals for at least the last ten days of life.

II. Experiments to determine the factors involved. In the second part of this research, an attempt was made to determine the factor or factors involved in the oestrual effect following total suprarenalectomy. In all the following experiments, the experimental rats consisted of only those females which definitely showed effects 1, 2 or 3 (see table 1) after both suprarenals were removed.

1. *Effect of transplants of adrenal cortex.* Nine experimental female rats showing complete oestrual inhibition for 9 to 12 days (at least two normal cycles) after complete suprarenalectomy were divided into groups of 3. Small pieces of suprarenal cortex varying in size from $\frac{1}{3}$ to $\frac{1}{2}$ were taken from the glands of normal rats and were transplanted into these females in the following locations: 1, intra-muscularly (dorso-lumbar and thigh musculature); 2, into the spleen, and 3, underneath the ovarian capsule. All members of the first two groups died of typical suprarenal insufficiency showing continued inhibition of oestrum. The 3 rats with intra-ovarial transplants survived, came back to normal weight and showed normal cycles (5–6 days) for at least 3 successive periods. Two of these females were bred and had normal litters of 7 and 8 respectively. By means of cortical transplants, normal ovarian function was restored. The ovary containing the cortical graft in these two females was removed three weeks after parturition. Both animals died 11 and 15 days later showing only one more cycle five days long.

2. *Effect of hypophyses of suprarenalectomized rats on immature ovaries.* The irregular occurrence of oestrual disturbance seen in table 1 suggested that the effect on the ovary is an indirect one. It was thought that possibly the hypophysis was the intermediary factor and that its gonad stimulating power in suprarenalectomized rats was decreased. The latter was tested by a method now commonly used, namely, implantation of the hypophysis into immature rats and weighing the hypertrophied ovaries.

A group of 36 suprarenalectomized male and female rats was used; the body weights of most of the animals and the vaginal smears of all females were recorded daily until definite signs of glandular insufficiency were seen. Rats in the last stages and others in approximately the midway period (animals with 9 per cent loss in body weight), were decapitated and bled to death. The hypophyses of two such animals of roughly the same body weight were then transplanted into the thigh musculature of an immature female rat (20–24 days old). The same procedure was followed with the pituitaries of 18 corresponding control rats. Five days after transplantation, the recipient females were killed and the two ovaries of each female were weighed together after the surrounding fat, connective tissue and oviducts were carefully removed. The following table summarizes the results of this experiment.

It is plainly seen in table 3 that total suprarenalectomy produces a definite and marked decrease in the gonad stimulating power of the pituitary gland. This effect is very marked in animals in the last stages of glandular insufficiency and is absent in suprarenalectomized animals that show a 9 per cent loss in body weight (roughly the mid-period of suprarenalectomy). The loss of gonad stimulating power of the anterior lobe of the hypophysis is greater in operated males and castrate males than in females. This is to be expected since the gonad stimulating content of the hypophyses of the former two is greater than in females.

TABLE 3

Effect of hypophyses of adrenalectomized adult rats on weight of immature ovaries

	NUMBER USED	NUMBER OF DAYS AFTER REMOVAL OF 2ND ADRENAL	AVERAGE WEIGHT OF IMMATURE OVARIES OF RECIPIENTS	PER CENT DECREASE IN GONAD STIMULATING POWER	REMARKS
			mgm.		
Control males	6		11.57		
Suprarenalectomized males	12	14–16 15.2	7.38	36.2	
Control castrate males	6		41.54		Males in castrate condition 4 weeks before experiment
Suprarenalectomized castrate males	10	12–15 14.3	26.67	35.7	
Control females	6		11.9		Only suprarenalectomized females showing effects 1 or 2 (see table 1) were used
Suprarenalectomized females	8	13–16 14.7	9.2	22.6	
Suprarenalectomized females in mid-period	6	10	12.1	No effect	

3. *Effect of injected hormones on the oestrual cycle of total suprarenalectomized female rats.* Following the results of the above experiment, it was natural to suppose that the effect of suprarenalectomy on the ovaries was by the way of the pituitary gland. If gonad stimulating hormone was supplied to female rats showing signs of suprarenal insufficiency and a complete inhibition of oestrum, would the ovary recover and oestrum occur? Would it recover following the injection of an ovarian hormone? These questions were answered by the results of 19 such experimental animals, 12 of which received injections of 0.5 cc. of Hisaw's gonad stimulating extract of powdered sheep pituitaries and 7 of which received

0.1 cc. or 1.5 rat units of theelin.[2] All injections were subcutaneous and were given twice daily for 3 to 5 days after the operated females showed complete oestrual inhibition for at least 12 days.

In every case, normal oestrum occurred as determined by the vaginal smear method. However, all 19 females showed fatal symptoms of glandular insufficiency and were exceedingly weak and unsteady 15 to 17 days after the removal of the 2nd suprarenal.

For control solutions, physiological saline and epinephrine chloride was injected subcutaneously into suprarenalectomized females showing similarly complete inhibition of oestrum for at least 12 days. The injections were carried out in essentially the same way —0.1 cc. of physiological saline and 0.5 cc. of epinephrine chloride (1:50,000) per injection given twice daily. Two groups of 6 rats each were used. All females died of suprarenal insufficiency with a continued oestrual inhibition.

4. *Cytological study of the hypophyses of adrenalectomized rats.* With some of the special stains and methods now available for studying the cytology of the anterior lobe of the hypophysis and depicting those cells presumably responsible for certain functions of this lobe, it was deemed advisable to save the hypophysis of some of the rats employed in each experiment. A detailed cytological study was made of this material by Doctor Bilstad and will soon be ready for publication. In short, it has been found that suprarenalectomy of adult males and females causes a decrease in the size and staining reaction of the oxyphil cells of the anterior lobe of the pituitary gland. The effect on the basophiles is not constant. Essentially the same change in the oxyphil cells occurs in the case of suprarenalectomized castrate males. It is concluded that complete suprarenalectomy produces definite cytological changes of the anterior lobe of the hypophysis.

5. *Effect of experimental loss of weight on the oestrual cycle of rats.* One of the well-known and most marked symptoms of suprarenal insufficiency is the gradual loss of body weight. It was believed that this symptom might be a contributory factor in the disturbance of oestrum following suprarenalectomy in addition to the absence of the cortical hormone. Perhaps with this weight loss there was also a serious decrease of vitamin intake (A, B and E). To test this, 14 female rats (135–170 grams) that showed at least three normal cycles, were placed in individual metallic cages and fed the ordinary Steenbock mixed diet. In addition, there were added twice per week 875 mgm. of dried powdered yeast (Northwestern Yeast Co.), 11 drops of cod liver oil, and a small piece of fresh lettuce. This special diet was always given first in tall dishes before the Steenbock mixture to insure complete ingestion and thus prevent any avitaminosis. Daily body weights and vaginal smears were recorded on all rats.

[2] Obtained by Dr. F. L. Hisaw from Parke, Davis & Co.

Of this group, two females were unilaterally suprarenalectomized and kept as controls and in two others, both glands were removed. Following this, the amount of Steenbock mixture fed to the remaining 10 unoperated females was decreased daily and was so graded that the loss in body weight almost paralleled the daily loss of the two doubly suprarenalectomized rats. The latter two died of typical glandular insufficiency in 13 to 14 days, one showing a complete oestrual inhibition and the other effect 2 (see table 1). The feeding of vitamins A, B, D and E to these rats showed no favorable effect on the ovaries. The per cent loss of body weight of these rats was 16.8; that for the group of 10 unoperated females ranged from 16.0 to 21.1 per cent (average 18.7 per cent). The unilaterally suprarenalectomized females showed a gain of 23.2 per cent in body weight for the same period. This large gain was undoubtedly due to the enriched diet (cod liver oil).

The results of the 10 non-adrenalectomized females given "the vitamins and decreased Steenbock diet" were striking. In 9 rats, the oestrual cycle remained uniform and undisturbed; in the remaining one, it was prolonged only one day which was in the 3rd oestrum of the 15-day period observation. Thus, it may be concluded that the gradual loss in body weight following double suprarenalectomy takes no appreciable part in oestrual disturbance.

Six of these ten animals were killed and their hypophyses preserved for cytological study. The other four were literally starved to death and seven days later were killed and the hypophyses, ovaries, uteri and vaginae saved. Just before death, these four animals showed an average fall in body weight of 37.8 per cent and postponed oestrum.

Discussion. Up to the present time there has been no uniform agreement in regard to the effect of total suprarenalectomy on the life of the white rat. A good deal of criticism has centered about the skill of the operative technique and the presence of accessory cortical tissue. The first factor can be controlled to a great extent so that after some practice, the technique, so far as tissue trauma is concerned, becomes negligible. Exceedingly short survival periods such as those reported by the early workers or even more recent ones (Freed, Brownfield and Evans) seem to us to point to operative procedures and to immediate postoperative effects. On the other hand, prolonged periods of 4 to 8 weeks and complete survivals are probably due either to the presence of accessory cortical tissue, which presumably takes over the function of the suprarenal cortex, or to failure in the first place to remove all of the gland with its pedicle and surrounding connective tissue. The latter is an important point since it has been shown by Pencharz and Olmsted and Pencharz, Olmsted and Giragossintz and others that small fractions of the cortex will proliferate and supply enough cortical hormone for the animal's economy.

It has been the experience of this laboratory following complete suprarenalectomies performed on more than 300 white rats of both sexes that these animals do not survive suprarenalectomy. This result is in accordance with the work of Pencharz, Olmsted and Giragossintz, Kutz and others and is true for both old and young rats. In the males used in this work, more deaths occurred (about 75 per cent) than one would ordinarily expect in view of the reported presence of accessory cortical tissue between the epididymis and the testis. The survival of female rats reported in table 1 is in part explained by the presence of accessory tissue confirmed histologically. However, in a few cases, no trace of accessories was found although the entire dorsal peritoneal wall especially in the mid-line from the diaphragm to the pelvis was carefully searched. Undoubtedly, cortical tissue was present and functioning in the body. The range in survival period for adult females was 7 to 27 days, the average being 15.2 days.

That the oestrual cycle in females white rats is disturbed following complete suprarenalectomy seems unquestionable. However, the effect is inconsistent and variable, which confirms the work of Wyman and Kitagawa. The most pronounced and consistent disturbances were seen in old multiparae and virgin females (8–12 months old). Perhaps in older animals, where presumably a higher level (in quantity) of hormonal activity prevails, a disturbance of endocrine equilibrium results in a sacrifice of the gonadal supply in favor of the more vital bodily processes.

In view of the inconsistent and variable effect on the ovaries, it seemed that the disturbance might be produced by some intermediary factor, perhaps the anterior lobe of the hypophysis. The results of the second part of this investigation support this idea. The mechanism involved seems to be a primary injury to the pars anterior following complete suprarenalectomy, which, in turn, is reflected on the ovary. This interrelationship of the pituitary and the adrenal cortex has been expressed by many workers who either studied clinical cases (Kraus, Moehlig and others) or who merely mentioned the idea in the discussion of related work. Smith made the first effective attempt to establish this relationship when he reported the pronounced decrease in the size of the adrenal cortex following hypophysectomy in rats. However, so far as the writer is aware, no experimental evidence has been published to show the result of total suprarenalectomy on the hypophysis. Such evidence would explain the irregular effect on the ovary noted in the first part of this paper and possibly the effect on the testis reported by Freed, Brownfield and Evans.

The suprarenal-pituitary relation is supported by the results of the second part of this paper. Successful cortical grafts (the ovary apparently being the most suitable location) restore normal body weight and ovarian

function. Removal of the graft results in the usual symptoms of glandular insufficiency and also disturbance in ovarian activity. Thus, we can be reasonably certain that normal ovarian function is in some way related and dependent upon the presence of the cortical hormone. The irregular occurrence of this disturbance as well as the varied effect naturally suggests the influence of some intermediary factor. This has been found to be the anterior lobe of the hypophysis on the basis of the following results: 1, decrease in the gonad stimulating power of hypophyses of suprarenalectomized rats (see table 3); 2, the cellular damage of such hypophyses seen in cytological preparations, and 3, the return of oestrum in suprarenalectomized females by injected extracts of the gonad stimulating hormone of the anterior lobe. When theelin was injected, another hormone present in normal ovarian activity that was apparently missing in suprarenalectomized females, normal oestrum also occurred. The results of injecting gonad stimulating hormone and theelin into experimental females merely show that an ovary will recover from an inactivated condition when the specific missing hormone is supplied. The injected material, however, did not prolong or save the life of the adrenalectomized rats. Injections of saline and epinephrine, as one would expect, were ineffective on the ovary and on the life of the operated females.

The cytological studies of hypophyses of experimental females further point to a functional glandular interrelationship with the suprarenal. When the period of post-suprarenalectomy was greatest, the cytological changes were most marked. Hypophyses of operated animals in the midway period (the point at which there is about 9 per cent decrease in body weight) showed little or no change. Undoubtedly, time is one of the essential factors. In short survival periods (9–12 days), it is easily possible that severe symptoms might intervene and cause the death of the rat before any possible pituitary disturbance could take effect.

It might well be argued that the ovarian effect following total suprarenalectomy is not at all specific and that factors such as temperature, loss of body weight, avitaminosis and surgical trauma might be responsible. This, of course, can be true when carried to extreme. However, all animals were given the same food, kept in the same more or less constant temperature room during the various seasons of the year and were operated on at different stages of oestrum with no appreciable effect on the oestrual cycle. Operative trauma is likewise of no effect as is seen in table 2. Experiments performed to determine the influence of possible avitaminosis and gradual loss of body weight (not exceeding 21 per cent) in unoperated females paralleling that of suprarenalectomized rats showed that both factors were negligible. The oestrual cycle strikingly remained uniform and undisturbed. Since the oestrual cycle was normal, it was concluded unnecessary to repeat the transplant experiment with

the pituitary gland of such experimentally starved rats. There is no reason to expect any significant decrease in the gonad stimulating power. From these feeding experiments, it is to be emphasized again that oestrual disturbance in suprarenalectomized rats is not due to avitaminosis since all the vitamins now regarded as necessary for normal oestrum were fed to operated females with negative results. The suprarenalectomized rats died and showed no return of oestrum.

SUMMARY AND CONCLUSIONS

1. Complete suprarenalectomy in the female white rat results in a suppression or irregular appearance of the oestrual cycle. This confirms the work of previous investigators. The effect, however, is inconsistent and occurs in only 87.6 per cent of the females.

2. It is also confirmed that the rat is no exception to the fatal symptoms of suprarenal insufficiency. In females, 80.1 per cent die from double suprarenalectomy.

3. Intra-ovarial transplants of suprarenal cortex maintain normal health and body weight of the rat. Pencharz and Olmsted's results are thus confirmed. These transplants also restore normal ovarian activity.

4. Complete suprarenalectomy in adult females, normal and castrate males, changes the cytological picture of the hypophysis of these rats and also produces a marked decrease in the gonad stimulating hormone of the anterior lobe.

5. Subcutaneous injections of theelin and of extracts of gonad stimulating hormone of the anterior hypophysis were effective in restoring oestrum although the females died from suprarenal insufficiency. Similar injections of normal saline and epinephrine chloride gave negative results.

6. The ovarian disturbance following complete suprarenalectomy is not due to operative trauma, avitaminosis (A, B, D, E) or to the gradual loss in body weight not exceeding 21 per cent.

7. In view of the inconsistent and varied effect on the ovary following double suprarenalectomy and the functional and cytological changes of the anterior lobe always coincident with marked ovarian disturbance, it is believed that an intermediate factor is present. This has been found to be the hypophysis and serves to explain the mechanism involved in the oestrual disturbance.

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PERIODIC VENTILATION AS INDUCED BY EXPOSURE TO HIGH PRESSURES OF OXYGEN

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Breathing an atmosphere low in oxygen quite commonly gives rise to periodic ventilation. In contrast to this are the results of experiments herein reported which were primarily designed for the study of oxygen poisoning in animals exposed to high pressure of oxygen. These experiments seem to be of particular interest, not alone because of the extremely long duration of the periodic cycles, but also because of the marked changes in volume flow of blood, blood pressure, pulse rate and blood color associated with the periodic fluctuations in ventilation.

The apparatus used was that described (Bean, 1930). The pressure chamber was supplied with an enclosed spirometer having accessory electrical apparatus which made possible the recording of respiratory ventilation on smoked paper. An especially constructed manometer was employed for the recording of the grosser changes in blood pressure. Due to its large column of mercury, the inertia was too great for the registration of the faster pulse rates. A small supplementary manometer enclosed within the chamber and provided with an electrical contact device in circuit with a recording signal magnet proved to be satisfactory. Volume flow of blood was recorded by the thermopile method as devised by Gesell and Bronk (1926). The glass thermopile vessel located outside of the pressure chamber made the observation of changes in blood color very convenient.

The oxygen used for these experiments was supplied from commercial pressure tanks. The chamber and spirometer were thoroughly washed out with fresh oxygen before each exposure. The pressures to which the experimental animals (dogs of about 10 kgm. weight) were exposed varied somewhat in the different experiments, but in most instances was about five atmospheres. This pressure, attained within a period of about four minutes, was maintained for varying lengths of time depending upon the reaction of the animal. Decompression was carried out in stages so as to eliminate the possibility of effects of effervescence which might arise from rapid decompression.

Prolonged exposure to high pressures of pure oxygen seems to have

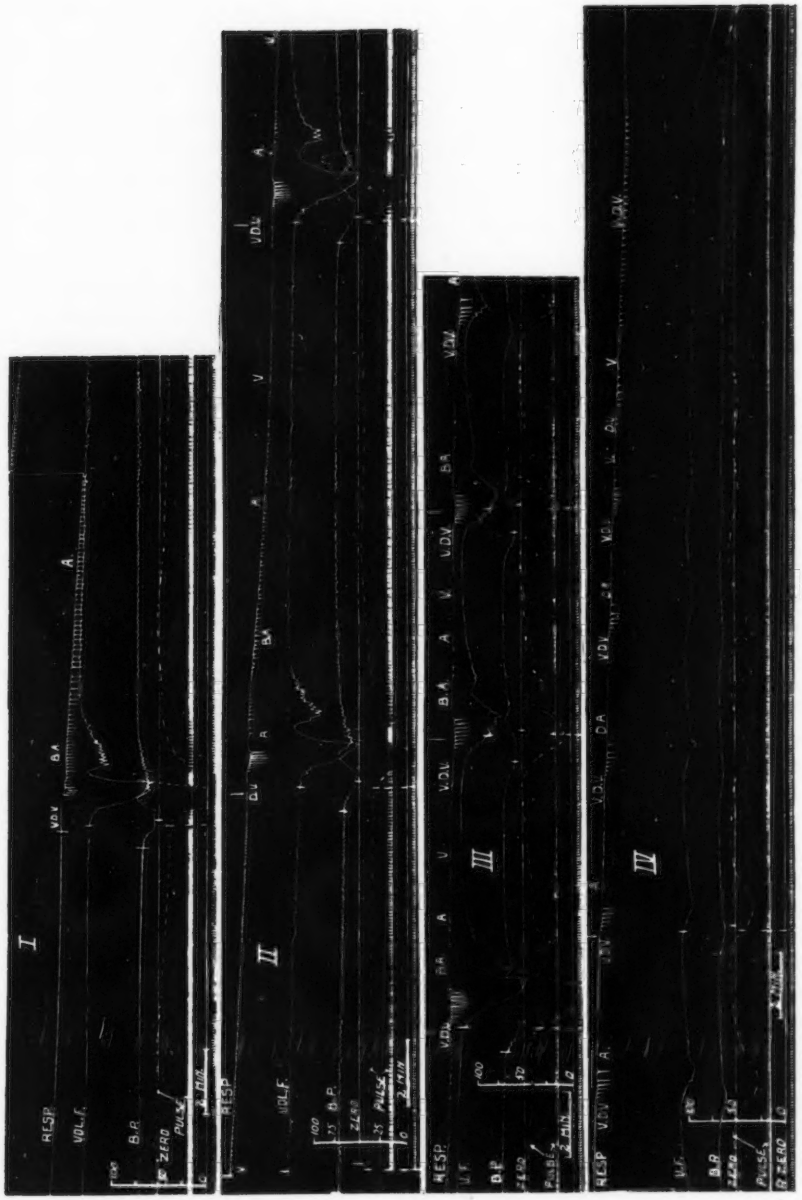
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Fig. 1

been a contributing factor in the appearance of this periodicity yet some of the longer exposures failed to initiate any of these cyclic changes. In some instances, periodicity began during the time at which the oxygen pressure was highest and disappeared on decompression. In others, it appeared during the early stages of decompression and continued some time after atmospheric pressure of pure oxygen had been reached. Occasionally periodicity began only after decompression to atmospheric pressure had been completed. Periodic breathing was also observed at 3 and 4 atmospheres of pure oxygen in unanesthetized rats by Gesell (1923) in his studies on high oxygen pressure (personal communication).

Figure 1 shows records obtained towards the end of an experiment in which the animal was exposed to 3700 mm. oxygen pressure for sixty five minutes, followed by decompression (over a period of ninety minutes) to atmospheric pressure of pure oxygen. During this decompression the respiratory minute volume decreased continuously from 600 cc. per minute per kgm. body weight at the height of exposure to complete apnea on reaching atmospheric pressure. This apneic period—a part of which is shown at the beginning of record I of figure 1—continued for fourteen minutes. During this interval the color of the arterial blood (carotid artery) flowing through the thermopile vessel turned to a very dark venous hue, indicated on the record as V.D.V. The arterial volume flow of blood remained at a relatively constant value and the blood pressure likewise showed no significant change from its low level of 40 mm. Hg until toward the end of this apneic period. The pulse rate (the broken line obtained by plotting the rate as recorded in the third line from the bottom) maintained a constant level for thirteen minutes then suddenly dropped to zero. There was a complete absence of pulse for forty-five seconds as indicated by the pulse record and direct inspection of the mercury meniscus. At the end of this time, the heart rate rose rapidly to almost twice that obtaining before the initial drop. The accompanying changes in blood pressure and volume flow of blood were equally abrupt, similar, and almost simultaneous. The blood color, too, changed rapidly as indicated on the record. The long apnea preceding these alterations was broken by deep ventilation which began when the volume flow of blood, blood pressure and heart rate were at a minimum and the blood extremely venous in color. The depth of this initial ventilation decreased with the sudden rise of the pulse, volume flow and blood pressure, and a return of the blood color to a bright artierial (B.A. in record). A secondary augmentation in ventilation occurred with the second decrease in blood pressure, heart rate and volume flow, only to diminish again as the volume flow, heart rate and blood pressure returned to steady high levels. A tertiary increase in depth of ventilation which also gradually diminished is to be noted from the record. The primary and secondary groups concur with decreases in blood pressure

and volume flow of blood and heart rate; the tertiary with a sustained increase in blood pressure and volume flow of blood. The color of the blood became less bright and gradually a dark venous color following the tertiary increase in respiration. The subsequent changes, shown in record II of figure 1 (the continuation of record I), are almost a duplication of those of record I. Here too, there are to be seen a primary, secondary and tertiary increase in respiratory minute volume. The tertiary augmentation decreased in this case to an apnea. The color changes also were essentially the same as those seen in the first cycle. About fifteen minutes after the beginning of this second cycle of changes a third series occurred, but from this point on (records III and IV) the intervals between the successive cycles of periodicity became shorter and the changes less abrupt. The tertiary increase in ventilation diminished and disappeared in successive cycles and then the secondary disappeared. The primary increase became more gradual in its onset and decreased in depth associated with less abrupt changes in blood pressure, volume flow, and heart rate. Toward the end of record III and throughout IV none of the cycles showed a complete absence of pulse. The blood color changes became less sharp with a tendency toward a continued venous color. The end of record IV indicates the death of the animal.

Further variations in periodicity are shown in records A, B, C, D, E, and F of figure 2, each occurring in a separate experiment. Record B was taken from an experiment in which the animal had been exposed to 3700 mm. oxygen for fifty-four minutes during which time there had been marked hyperpnea. The pressure was lowered in three stages over a period of thirty minutes to 2068 mm. pressure of oxygen—the pressure at which the record was obtained. An apneic period of twenty minutes preceded the first ventilation shown in record B. This first period of increased ventilation occurred during a decreased volume flow of blood and when the heart rate and blood pressure were, so far as could be determined, zero; a second, and more prolonged period of ventilation was accompanied by an increased flow of blood, heart rate and blood pressure. The picture here resembles very closely some of the cycles shown in figure 1. In this experiment, as in figure 1, there was a very low general blood pressure—20 mm. was the highest value. The respiratory groups correspond to those in figure 1, spoken of as the primary and tertiary increases in respiratory ventilation. The primary occurred during a decreased volume flow and blood pressure, the tertiary during an increased flow and blood pressure.

In record D, figure 2, periodic changes came on when the oxygen pressure had been lowered from 3600 mm. to 1760 mm. Hg. The apnea in this case occurred during the periods of increased heart rate and blood pressure. Volume flow was not followed. The general level of blood pressure was

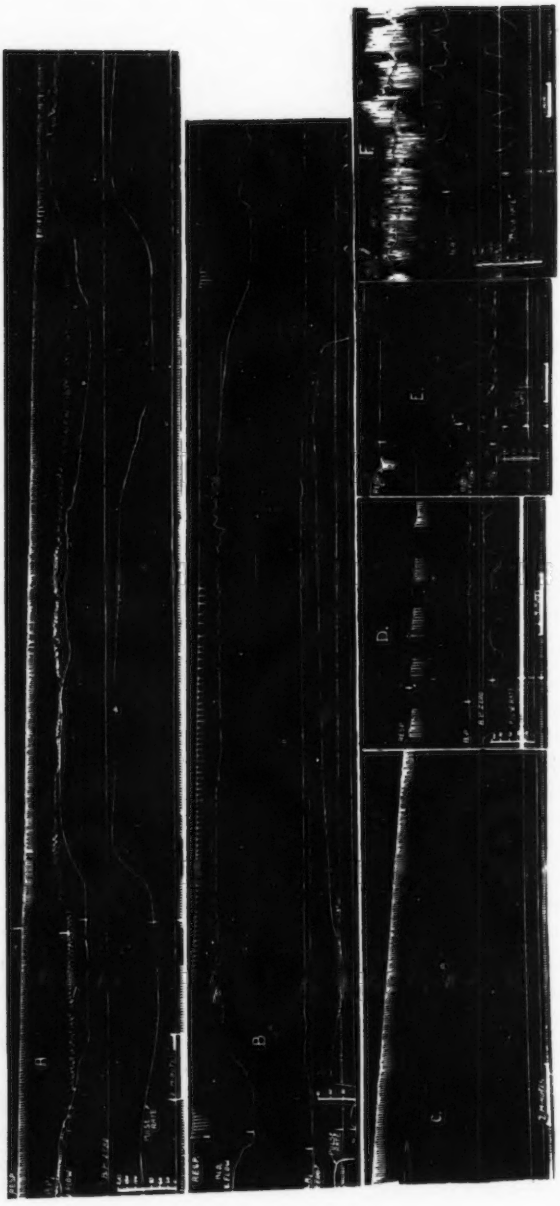

Fig. 2

only 36 mm. Hg. This periodicity corresponds essentially with that seen in record IV of figure 1 and is of a relatively simple variety.

Record E, figure 2, was obtained from an experiment in which the animal was exposed for thirty-five minutes to a pressure of 3680 mm. pure oxygen and after sixty minutes of decompression in three stages to 1000 mm. Hg. Apnea had come on at this pressure and had continued several minutes. The fourth stage of decompression was begun, but was only carried to 950 mm. Hg—indicated by the rise in the spirometer line at the beginning of the record (E, fig. 2). The blood pressure, volume flow and heart rate were decreasing when this reduction was made. The decrease in heart rate continued to a complete cessation as far as could be determined. Respiratory movements appeared during the period of cessation of pulse and decreased blood flow and blood pressure. The sharp rise in pulse rate and volume flow was accompanied by an apnea which continued to death ten minutes later. It is interesting that while respiration showed no further periodicity, marked periodic changes in pulse, blood pressure and blood volume flow continued to death. The heart, periodically breaking through the inhibition, apparently supplied enough blood to the cardiac center to maintain its function. The respiratory control mechanism, however, had apparently fallen to such a low level as to be incapable of further response. It should be noted that periodicity in blood pressure, heart rate and volume flow of blood preceded ventilation. This is another instance in which periodicity occurred during a very low blood pressure—the highest in the record was 24 mm. Hg and here, too, apnea was broken by ventilation which began during a period of decreased heart rate, blood pressure and volume flow of blood.

Record A, figure 2, was taken from an experiment in which the periodic cycles in ventilation, volume flow of blood and blood pressure occurred during exposure to 3700 mm. pressure of oxygen. These periodic changes (record A, fig. 2) were of about eight minutes' duration. No apnea occurred, but rather a relative decrease in ventilatory depth and rate during which time the pulse rate, volume flow of blood and blood pressure were lowest. This periodicity appeared after thirty minutes' exposure to the increased pressure and lasted until the first stage of decompression (about two hours later) at which point it became less distinct, but did not disappear entirely. It is of interest to note that during this periodicity shown in record A the blood pressure (120 mm. Hg) was considerably higher than that in the preceding experiment. Also the blood remained uniformly bright arterial. With later decompression and after breathing oxygen at 2300 mm. for forty minutes apnea came on and lasted twenty minutes. The blood pressure began to fall slowly and for fear of possible loss of the animal the oxygen pressure was raised to 2700 mm. Two breaths were taken and volume flow increased slightly, apnea followed for eight minutes

and blood flow began to fall again. The oxygen pressure was then lowered to 1500 mm. Hg. At a low point in blood pressure and volume flow ventilation returned and again showed periodicity, but of a different type than that which occurred at 3700 mm. pressure. The sequence of the cycle here corresponded closely to that shown in record III of figure 1. This periodicity continued for forty minutes during which time the pressure was lowered in one stage to 960 mm. Death occurred at this pressure.

The effect on the respiration of another animal which had been exposed to 3700 mm. pure oxygen is shown in record C, figure 2. This periodicity began fourteen minutes after raising the pressure and continued for one and one-half hours and through five stages of decompression which took another hour and fifteen minutes. The portion shown in C was recorded at a pressure of 2600 mm. Hg—one of the stages in decompression. This periodicity shows no apneic periods and the waves are relatively long, resembling those of record A, figure 2.

Record F represents still another experiment in which an animal had been exposed to pure oxygen at 3800 mm. Hg pressure for twenty-four minutes. The periods of decreased respiratory minute volume occurred during increased pulse rate, blood pressure and blood volume flow. There were no complete apneic periods but the decrease in respiratory minute volume was attended by a very shallow, rapid, panting type of ventilation. The rise in the level of the respiratory record in F was due to decompression to 2500 mm. Further decompression caused a shortening of the periodic changes and later a complete disappearance. In this instance the blood pressure was relatively high (130 mm. Hg at the lowest point) throughout, but the ventilation was quite unlike that seen in the other experiments in which periodicity was associated with a high blood pressure.

Discussion. The periodicity in respiratory ventilation shown in these records might be classified for purposes of discussion as being of two types which simulate in some respects those observed under different experimental conditions by Eyster (1906). The first, and probably more common variety, is exemplified in record IV of figure 1. Here the general level of blood pressure was low and increased ventilation concurred with a decrease in blood pressure, heart rate, and volume flow of blood. Each successive respiratory increase failed to bring about any sustained improvement in the animal, as indicated by the blood pressure, and death soon followed. A similar response is shown in record D of figure 2 which is from another experiment. The second type of periodic respiration is illustrated in record A of figure 2 where the period of increased respiratory ventilation was concurrent with an increased pulse rate, blood pressure, and volume flow of blood. The general level of blood pressure in this case was high and there was no apnea. Were the discussion to be confined to these records the conclusion that the two types of periodic respiration were

related to different physiological states of the animal would be justified. Casual inspection of records I and II of figure 1 might seem to invalidate such a conclusion since in these records there is a combination of the two types of periodicity occurring with what, at first sight, appears to be a common low condition of the animal. Careful study of the records, however, reveals that there were periods of improvement—more or less sustained—during which periodic respiration of the second type prevailed.

During the long apneic period (record I, fig. 1) the carotid blood had become very dark venous. This may have given rise to a vagal stimulation and a weakening of the heart resulting in a sharp fall in blood pressure and volume flow of blood. This sudden reduction in blood pressure and volume flow of blood in all likelihood led to a sudden increase in the stimulation of a depressed respiratory neuro-mechanism and brought about the initial or primary respiratory ventilation. This ventilation apparently improved the sensitivity of the controlling mechanism—or shifted the control to another level since the secondary increase in respiration occurred with a smaller fall in blood pressure, volume flow and heart rate. This secondary respiratory increase must in turn have brought about still further improvement in the condition of the animal since the blood volume flow and blood pressure subsequently rose to values higher than obtained during any preceding point of the record. The changed color of the blood also indicated an improved condition. It was during this improvement (which was sustained for some minutes) that the tertiary respiratory increase—periodic breathing of the second type—occurred. For some reason this improved physiological state was not maintained indefinitely and the animal slipped back to its former lower level. The series of events shown in record II was similar to that of record I. This second attempt at a prolonged recuperation ended like the first and when record III was reached the periodic respiratory response became purely one of the first type and continued through record IV to the death of the animal. With this disappearance of the tertiary increase in respiration, the sustained increases in blood pressure and volume flow also disappeared and there was a gradual drop in values in each successive cycle.

A somewhat similar response of another animal is shown in record B figure 2. In this instance, the secondary respiratory increase was absent, but the combination of the two types of periodic respiration might be explained in a manner similar to that above.

Analysis of these records showing a combination of the two types of periodicity then supports rather than disproves the belief that each was associated with a different physiological state of the animal. Either the respiratory controlling mechanism was behaving differently under the different conditions or there are different control mechanisms for these various conditions.

One of the puzzling observations in many of these experiments was the striking color changes accompanying periodic changes in respiration, heart rate, blood pressure and volume flow of blood. In an animal breathing pure oxygen it would seem as though the arterial blood would remain uniformly arterial in color. The lungs at the beginning of the long apneic period of record I, figure 1, were presumably full of oxygen—certainly not enough nitrogen could have come from the animal and accumulated in the lungs and spirometer during the experiment to be of any significance. The spirometer had been adequately washed out and previous tests had shown it to be free from carbon dioxide. The blood, while maintaining a relatively constant flow, gradually darkened in color to the end of the apnea at which time it had reached an extreme degree of venosity. In all likelihood the animal was then in a state of asphyxia. In support of this view is the marked slowing of the pulse—even to cessation—an effect quite commonly seen in asphyxia. Any rise in blood pressure which would have occurred as a result of asphyxial vasomotor constriction was overshadowed by the cardiac reaction resulting from stimulation of the vagus center and perhaps a weakening of the heart itself. This venosity of the blood was most probably due to a depletion of the oxygen in the lungs during the long apneic period.

Using figures based on human metabolism the basal consumption of oxygen in a 65 kgm. man of 170 cm. height with an R.Q. of 0.8 is found to be 14.5 liters per hour or 3384 cc. in fourteen minutes (DuBois, 1927). With the lungs filled with pure oxygen there would be nearly complete depletion of the oxygen at the end of this period. If we assume that the same relation of lung capacity and metabolism is roughly approximated in the dog, the extreme venosity at the end of the fourteen minute apnea is explained.

Dautreband and Delcourt-Bernard (1928) suggest that a constriction of the capillaries of the brain was a contributing factor in the appearance of the Cheyne-Stokes respiration they observed during administration of pure oxygen at atmospheric pressure. In view of the changes in blood color and volume flow of blood it is not likely that such was a contributing factor in the periodicity seen in the present experiments.

The periodic changes which were observed in our experiments were without exception attended by alterations in heart rate, whether the periodicity in ventilation involved apnea or not. Greeley and Greeley (1930) found that periodic breathing without apnea may also occur in the absence of alteration in heart rate.

SUMMARY

Exposure of animals to high barometric pressure of pure oxygen may give rise to periodic breathing, periodicity in heart rate, blood pressure, volume flow of blood and blood color changes.

The periodicity observed was especially striking in that there occurred, quite frequently, very long apneic periods—twenty minutes in some cases, complete cessation (so far as was determinable) of pulse with accompanying fall of blood pressure to zero and abrupt decreases in volume flow of blood. The color of the carotid blood changed periodically from a bright arterial to a very dark venous hue.

It was found that this periodicity might appear at the height of exposure to the increased pressure and subside on decompression. It often appeared in some stage of decompression or after the pressure had been lowered to one atmosphere of pure oxygen.

The periodic ventilation was of two types, one in which the ventilation was increased during a fall in blood pressure, volume flow of blood, and slowed heart rate,—a second in which the augmentation in ventilation occurred during a period of increased volume flow of blood, heart rate and blood pressure.

It is suggested that the two types of periodicity were perhaps due to difference in activity of the respiratory controlling mechanism or activity of different parts of the same mechanism under different physiological states of the animal involving flow and blood pressure.

Computation indicates that the changes of color which occurred in the blood were primarily a result of a depletion of the store of oxygen in the lungs giving rise to asphyxia.

The broken coördination of the dual function of the hemoglobin due to high oxygen pressure or asphyxia may play a rôle in the production of periodicity as observed in these experiments.

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A COMPARISON OF THE RESPONSE OF THE ANESTHETIZED DOG TO LOWERED ALVEOLAR OXYGEN DURING UNIFORM ARTIFICIAL VENTILATION AND DURING NORMALLY CONTROLLED VENTILATION[1]

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In an earlier series of experiments (Gesell, Krueger, Gorham and Bernthal, 1930) various respiratory phenomena were studied under numerous conditions, such as lowered alveolar oxygen, intravenous injection of sodium cyanide, hemorrhage, intravenous injection of sodium bicarbonate, hydrochloric acid, methylene blue, etc. Among the factors studied were carbon dioxide and oxygen content of expired air, total elimination of carbon dioxide and total absorption of oxygen, expiratory quotient, lactic acid content of the blood, carbon dioxide capacity of the blood, hydrogen ion concentration of the blood, volume flow of blood, mean arterial blood pressure, oxidative energy, non-oxidative energy, the relation of shortage of oxygen consumption to accumulated lactic acid, the relation of excess oxygen consumption to the disappearance of lactic acid, the relation of total elimination of carbon dioxide to that required by oxidations, the relation of the lactic acid content of the body to the liberation and retention of carbon dioxide, the proportion of fixed acid combining with bicarbonate and the proportion reacting with other buffer systems. In all of these experiments pulmonary ventilation was maintained at a known uniform volume.

We have now made a similar study during administration of gaseous mixtures low in oxygen and during intravenous injection of sodium cyanide, but in the present experiments the chest was kept intact and pulmonary ventilation was under the control of the respiratory mechanisms of the animal. The animal was, therefore, capable of controlling to some extent the oxygen and carbon dioxide pressure of the alveoli and tissues. Obviously the results might differ in very essential details from those in which ventilation was uniform.

Data of three experiments on three different dogs under morphine urethane anesthesia are presented in figures 1, 2, and 3 and tables 1, 2, 3, and 4. In one experiment a 7.24 per cent oxygen mixture was administered for

[1] Preliminary report: Proc. Soc. Exper. Biology and Medicine, 1930, xxvii, 849.

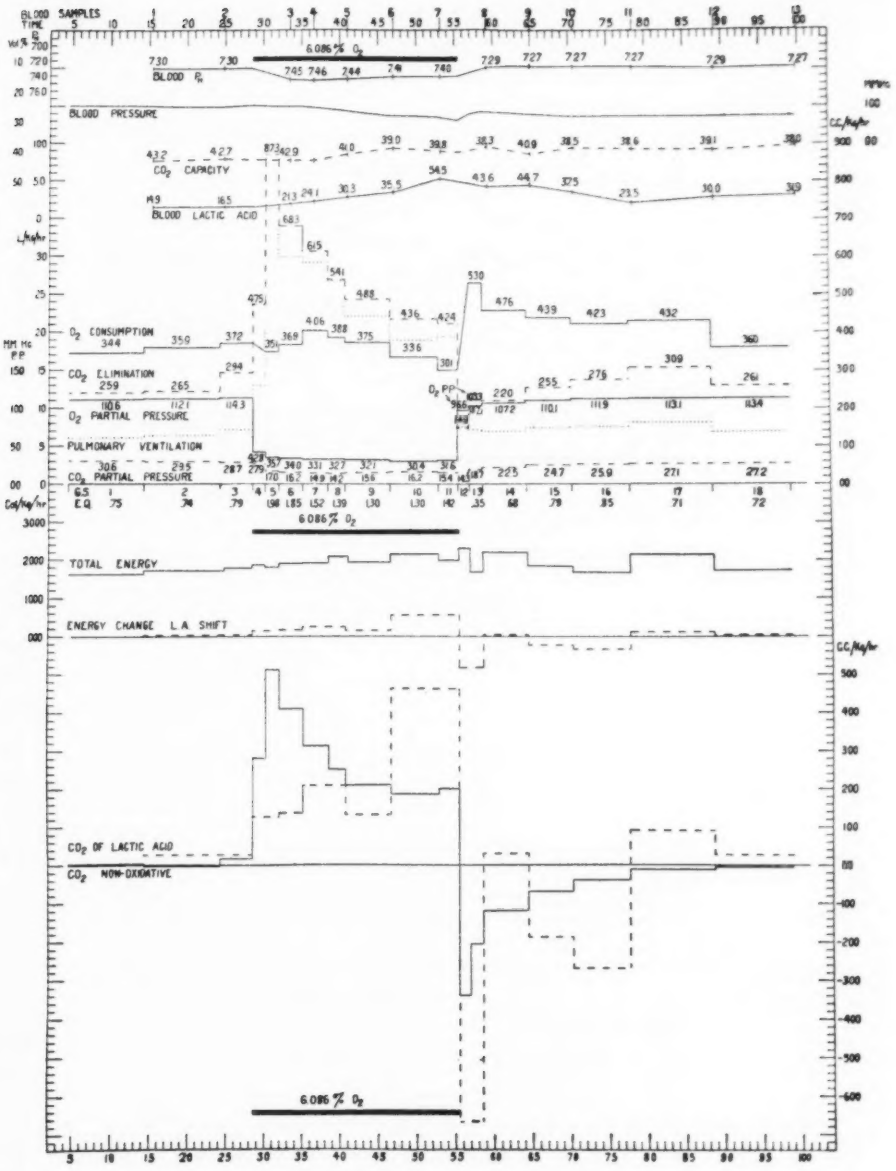

Fig. 1. Experiment 1, May 6, 1930

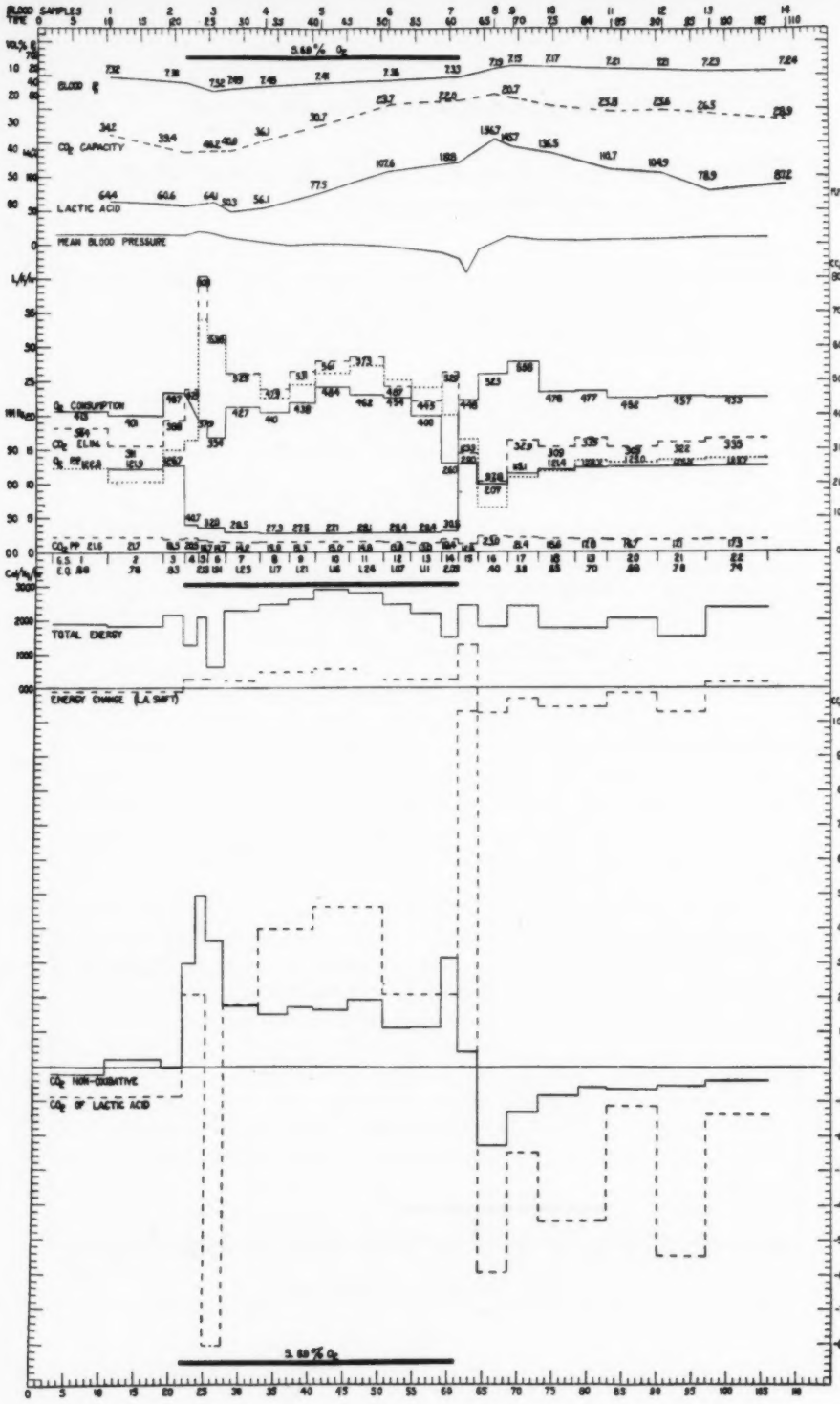

Fig. 2. Experiment 2, June 2, 1930

25 minutes and 8 seconds, in another 6.09 per cent oxygen mixture was administered for 26 minutes and 48 seconds and in the other a 5.69 per cent oxygen mixture was administered for 39 minutes and 50 seconds.

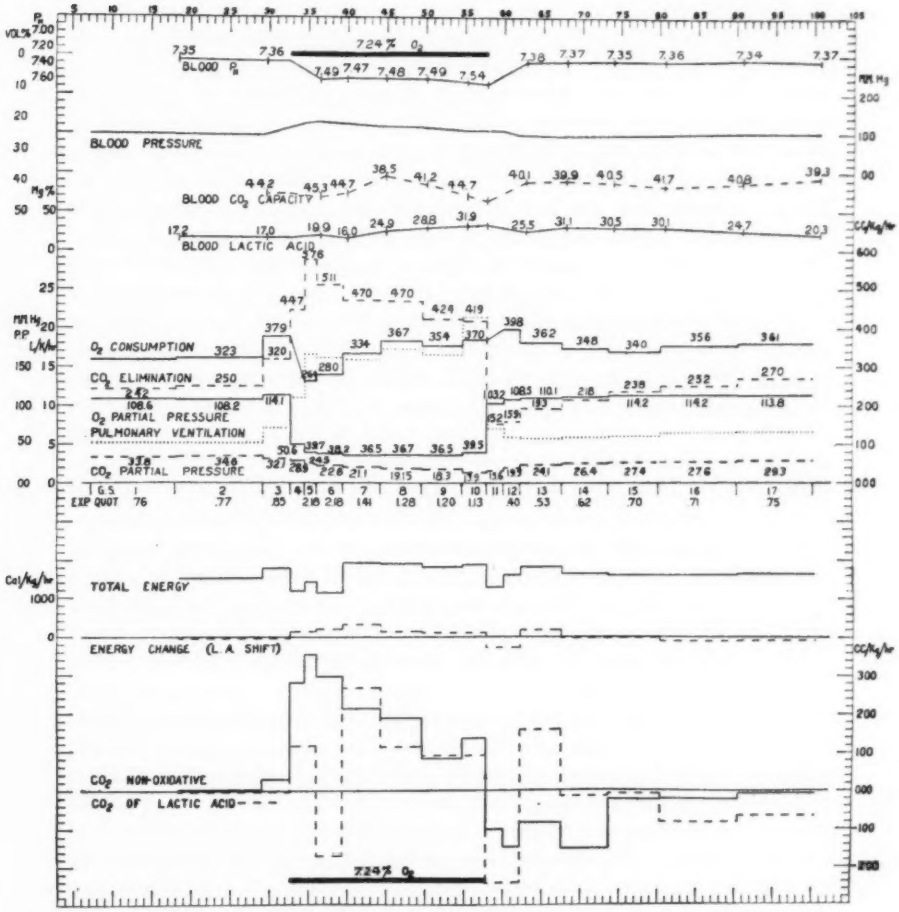

Fig. 3. Experiment 3, April 29, 1930

The total expired air was collected in rubber bags of suitable size connected by a three-way valve with the expiratory outlet of an inspiratory-expiratory valve. After allowing the collected samples to come to room temperature, two 50 cc. samples were withdrawn for analysis and the

volumes of the expired samples determined with a gas meter. For methods of computation and analysis the preceding papers should be consulted.

Experiment 1. Administration of the 6.09 per cent oxygen mixture led to a drop of expired oxygen pressure in the first two minutes from 112.1 mm. Hg in G. S. 2 to 35.7 mm. Hg in G. S. 5. The pressure then fell more slowly to a minimum level of 30.4 mm. in G. S. 10. During the initial period of lowered alveolar oxygen, pulmonary ventilation increased about five-fold, from 7,000 cc. per kgm. hour in G. S. 2 to about 36,500 cc. in G. S. 5. After the first three minutes of augmented ventilation the movement of air decreased again, but at the end of the period of lowered alveolar oxygen it was still approximately three times the preadministration ventilation. Oxygen consumption was not markedly affected—it first decreased, then increased above normal and finally decreased again below basal values. During this period of augmented ventilation the carbon dioxide pressure of expired air decreased from 29.5 to 14.2 mm. Hg. The curve of carbon dioxide elimination shows an increase from 265 cc. per kgm. hour while breathing room air, to 873 cc. during the initial period of augmented ventilation. Elimination then fell off along with pulmonary ventilation to 424 cc. per kgm. hour. The abrupt increase in carbon dioxide elimination occurring at the beginning of the period of lowered alveolar oxygen was associated with a corresponding abrupt increase in the expiratory quotient from 0.74 to 1.98. As ventilation diminished the expiratory quotient receded to somewhat lower levels (1.30, 1.30, and 1.42), but was still considerably higher than the preadministration values. The greater expiratory quotient, most probably, is primarily a resultant of three variables—increased pulmonary ventilation, augmented cardiac output and increased formation of lactic acid. Note the curve of carbon dioxide equivalent of lactic acid and compare this with the curve of non-oxidative carbon dioxide elimination.

It will be seen that lowered alveolar oxygen led to an immediate increase in the lactic acid content of the blood. This increase was maintained throughout the entire period despite the fact that oxygen consumption was temporarily increased. The increase in blood lactic acid may perhaps in part be attributed to the lowered hydrogen ion concentration of the blood (Macleod, 1921). On the other hand excessive oxygen consumption is by no means indicative of an adequate supply of oxygen. As a matter of fact the record indicates that the animal was deteriorating though oxidations were relatively high, for toward the close of the period of lowered alveolar oxygen, oxygen consumption again fell below the normal basal levels. Lactic acid accumulation was then augmented still more. Comparing the curves of carbon dioxide capacity and lactic acid content of the blood, it will be seen that the carbon dioxide capacity of the blood diminished at a rate slower than the accumulation of lactic acid, indicating as in the preced-

ing series of experiments that only part of the lactic acid reacts with bicarbonate base. Despite the accumulation of lactic acid and reduction in bicarbonate buffer base the blood turned more alkaline to the extent of 0.16 pH. The disproportionate blowing off of carbon dioxide and the lowered oxygenation of hemoglobin appear to be the main contributing factors to this effect. The further increase in blood lactic acid and the lowered ventilation must have contributed to the subsequent increase in hydrogen ion concentration from pH 7.46 to 7.40.

With readministration of room air there was a general reversal of conditions. During a period of two gas samples (13 and 14) pulmonary ventilation fell to the preadministration level and thereafter remained on the whole somewhat above the normal level. The increase in alveolar oxygen pressure was accompanied by an immediate overshooting of oxygen consumption, well sustained, but most pronounced in samples 12, 13, and 14. This period of greatest oxygen consumption was accompanied by the lowest oxygen pressure of the expired air (96.6, 103.3 and 107.2 mm. Hg) occurring during administration of room air and is accounted for by the subnormal ventilation and the supernormal absorption of oxygen. As pulmonary ventilation increased and oxygen consumption diminished the pressure of expired oxygen again equalled that of the preadministration period.

With readministration of room air the carbon dioxide pressure of the expired air fell from 15.4 to 14.3 mm. Hg and carbon dioxide elimination dropped from 424 cc. per kgm. hour to 149. From this point on, both the partial pressure of expired carbon dioxide and the total elimination of carbon dioxide increased to superbasal levels. The changing relations of oxygen consumption and carbon dioxide elimination which are easily followed in the graph led to rapid changes in the expiratory quotient. The most pronounced change occurred from gas sample 11 to gas sample 13 when the quotient dropped from 1.42 to 0.35. Except for gas samples 15 and 16 the expiratory quotient was lower than the normal preadministraion values. Since the lactic acid of the blood is seen to diminish on readministration of room air, the reduction in the expiratory quotient is conceivably due to the the retention of carbon dioxide by the base liberated in the disappearance of lactic acid as well as to the reduction of pulmonary ventilation and absorption of carbon dioxide by the desaturated tissues.

On readministration of room air the hydrogen ion concentration of the blood increased above normal despite the previous hyperelimination of carbon dioxide and despite the hypernormal ventilation. The persistently higher lactic acid values are of interest in this connection.

In experiment 2 a gaseous mixture with a lower oxygen content was administered and some of the effects were correspondingly greater. With the fall in oxygen pressure of the expired air from 121.9 mm. Hg at gas

sample 2 to 32.0 mm. at gas sample 5, pulmonary ventilation increased 226 per cent from 15.02 liters per kgm. hour to 34.12 liters. This increase will be seen to be less than in the preceding experiment despite the lower oxygen pressure of the administered gas. As in experiment 1, augmentation of ventilation was the greatest at the beginning of the period of lowered alveolar oxygen. This was followed by a decrease up through sample 8 and a second increase with the maximum during gas sample 11. The oxygen pressure of the expired air after reaching the low value of 27.3 mm. Hg at gas sample 8 remained at a relatively constant value. Oxygen consumption again showed an initial fall to 334 cc. per kgm. hour in gas sample 6 after which it increased to supernormal levels reaching a maximum of 484 cc. per kgm. hour in gas sample 11. From that point on to the end of administration, oxygen consumption rapidly fell to 260 cc. per kgm. hour. The fall in carbon dioxide pressure, beginning with lowered alveolar oxygen, was associated with an augmented elimination of carbon dioxide running roughly parallel with pulmonary ventilation. The expiratory quotient rose to the high level of 2.13 to fall again to 1.07 and 1.11 in gas samples 12 and 13. At the very end of the period of lowered alveolar oxygen the quotient rose again to 2.03. This final increase appears to be due to the sudden collapse of the animal associated with a reduction in oxygen absorption and an absolute increase in carbon dioxide elimination. The initial decrease in the hydrogen ion concentration of the blood during the period of lowered alveolar oxygen to pH 7.2 can, as in experiment 1, be attributed to increased pulmonary ventilation, increased volume flow of blood and decreased oxygenation of the blood. The subsequent large increase in the hydrogen ion concentration of the blood amounting to 0.19 pH is accompanied by an increase in lactic acid content of the blood amounting to 55 mgm. per 100 cc. of blood. Again the changes in carbon dioxde capacity of the blood were not proportionate to the changes in lactic acid content of the blood.

With readministration of room air pulmonary ventilation fell distinctly below normal at gas sample 16. Though oxygen consumption more than doubled, carbon dioxide elimination was reduced to less than one-fifth the elimination in gas sample 14. There was a corresponding drop of the expiratory quotient from 2.03 to 0.40 after which there was a slow return to 0.74 which is still below the preadministration level. This prolonged subnormal expiratory quotient is probably related to the rapid disappearance of lactic acid as indicated in the lactic acid curve. The rapid disappearance of lactic acid is accompanied by a reduction in the hydrogen ion concentration from pH 7.15 to 7.24. This final concentration is still higher than the normal and corresponds to a relatively low carbon dioxide content of the blood. The disproportionate change in the carbon dioxide capacity and lactic acid content of the blood is again very definite.

The results of experiment 3 differ in a number of points from those of experiments 1 and 2. While pulmonary ventilation was increased by the administration of gaseous mixtures low in oxygen the sudden high initial increase was entirely missing. In its place there was only a slight augmentation which increased progressively up to the very end of lowered alveolar oxygen. Oxygen consumption showed the customary fall at the beginning of administration, also the secondary increase, but the final decrease noted in experiments 1 and 2 was missing. Apparently the animal was reacting very well to the exposure to low oxygen. In observing the curve of carbon dioxide elimination it is interesting to note that despite the small initially increased ventilation, the early maximum carbon dioxide elimination still obtained and as ventilation increased carbon dioxide elimination decreased as it did in the other experiments. Another striking difference in results is the very small increase in blood lactic acid. This, along with the low initial value, is indicative of a general vigorous condition of the animal. The changes in blood carbon dioxide capacity are also worthy of note. First the carbon dioxide capacity decreased out of proportion to the increase in blood lactic acid and then rapidly increased to supernormal values despite the fact that lactic acid was increasing in the blood. This peculiar phenomenon, though not as pronounced, is clearly evident in experiment 1 and indications of it are also seen in experiment 2. The results are suggestive of an outpouring of base from some carbonate depot such as the bones or the outpouring of red blood cells from the spleen as protective measures against acid tendencies of anoxemia. It will be seen that the blood turned distinctly alkaline in this experiment and instead of showing the usual tendency of increasing acidity of experiments 1 and 2 the blood continued more alkaline throughout low oxygen exposure. To be sure, this well maintained alkalinity may have been due to the increasing pulmonary ventilation as well as to the addition of base to the blood.

When a dog is subjected to an atmosphere of lowered oxygen pressure various changes which occur may conceivably be adjustments conducive to the well being of the organism. Perhaps the outstanding of these are increased circulation and ventilation of the blood. The first is usually associated with a faster rate of heart beat and ejection of blood against a higher pressure. The second is accompanied by a greater movement of air in and out of the respiratory passages, a warming of the greater volume of air, the saturation of this greater volume of air with water vapor and the liberation of a greater amount of carbon dioxide from the blood into the alveolar air. All of these processes are energy consuming. If a continuation of the normal basal rate of dissipation of energy is essential for the continuance of normal basal function it is of interest to determine, if possible, to what extent the demands for energy, basal and superbasal are met under conditions of oxygen stress.

Some of these energy relationships are summarized in table 1, where all values are represented in equivalents of energy in terms of oxidations in cubic centimeters of oxygen (1 cc. of oxygen = 4.8 calories). Each experiment is divided into three periods, the period of low oxygen, the period of recovery and the combined period of low oxygen and recovery. The first line gives the expected basal oxygen consumption assuming a continuation of the basal rate during the preceding period of administration of room air. A comparison of the expected and the observed oxygen consumption during the period of lowered alveolar oxygen shows a small balance in favor of excess oxygen consumption above the basal require-

TABLE 1

Energy relationships

	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3		
	Low O_2 G. S. 4-11	Recovery G. S. 12-18	Low O_2 and recovery G. S. 4-18	Low O_2 G. S. 4-14	Recovery G. S. 15-22	Low O_2 and recovery G. S. 4-22	Low O_2 G. S. 4-10	Recovery G. S. 10-17	Low O_2 and recovery G. S. 4-17
1. Basal O_2 consumption	151.2	260.9	412.1	270.1	303.2	573.3	134.4	226.3	360.7
2. Observed O_2 consumption	151.5	308.5	460.0	271.9	345.0	616.9	138.2	250.9	389.1
3. Oxygen balance	+0.3	47.6	47.9	+1.8	+41.8	+43.6	+3.8	+24.5	+28.3
4. Anaerobic energy	+28.9	−19.3	+9.6	+40.2	−22.0	+18.2	+10.7	−8.5	+2.2
5. (3) + (4)	+29.2	+28.3	+57.5	+42.0	+19.8	+61.8	+14.5	+16.0	+30.5
6. O_2 work (ventilation)	−0.3	−0.1	−0.4	−0.4	−0.0	−0.5	−0.1	−0.0	−0.1
7. O_2 warming air	−5.8	−1.5	−7.3	−7.2	−0.8	−8.0	−3.7	−0.6	−4.3
8. O_2 saturating air	−22.4	−5.7	−28.1	−27.8	−3.2	−31.0	−14.5	−2.4	−16.9
9. O_2 CO_2 liberation	−11.9	+3.5	−8.4	−13.1	+5.7	−7.4	−8.3	+3.9	−4.4
10. (6) + (7) + (8) + (9)	−40.4	−3.8	−44.2	−48.5	+1.7	−46.9	−26.6	+0.9	−25.7
11. (5) + (10)	−11.2	+24.5	+13.3	−6.5	+21.5	+14.9	−12.1	+16.9	−4.8

ments, but much too small to meet any substantial demands for circulatory and respiratory adjustments. On the other hand, without circulatory and respiratory adjustments oxygen consumption would undoubtedly have fallen far below normal as has been established in our earlier experiments in which artificial ventilation of uniform volume was administered (Gesell, Krueger, Gorham, and Bernthal, 1930). During the period of recovery however oxidations increased and the oxygen balance was definitely in favor of superbasal oxidation to the extent of 47.6, 41.8, and 24.5 cc. For the combined periods the balance was in each case slightly larger—47.9, 43.6, and 28.3 cc. Assuming that the energy equivalent of this excess

oxidation is available for superbasal activities of adjustment and recovery we have added, whether correctly or not we do not know, the anaerobic energy dissipated as a result of liberation of lactic acid which brings the total extra dissipated energy above the basal requirements in terms of oxygen to 57.5, 61.8, and 30.5 cc.

The computed energy required to meet the various altered conditions follow next. Accepting Rohrer's (1925) formula ($W = 0.6\ L^2$ kgm. = $0.003^-\ L^2$ cc. of O_2 per minute where W is the energy equivalent of the work and L the minute volume) for determining the work required to move the extra air of superbasal ventilation the oxygen requirements are seen to be very small—about 0.4, 0.5, and 0.1 cc. per kilogram hour respectively. (The computations are based on a uniform respiratory rate of 15 per minute.) The energy required for warming the air of superbasal ventilation is greater. Assuming a change in temperature from inspired air at 20°C. to expired air at 32°C. (these temperatures were not followed during the experiments) the energy equivalent of warming the air is 7.3, 8.0 and 4.3 cc. The energy requirements for the heat of liberation of the superbasal carbon dioxide from the blood into the alveolar air are of a similar magnitude, 8.4, 7.4, and 4.4 cc. of oxygen respectively. ($NaHCO_3$ +HP-NaP + H_2O + CO_2 + 10,850 cal. P represents protein anions. Hence the movement of 1 cc. of carbon dioxide into or out of the body involves 0.484 calory or the energy equivalent of 0.101 cc. of oxygen. Derived from the equations of Meyerhof, 1925.) Saturation of the air with water vapor appears to be of the most significant item of all the superbasal requirements. Computing for a saturation from 50 per cent at 20°C. to 100 per cent at 32°C. (Liljestrand and Sahlstadt, 1924) 28.1, 31.0, and 16.9 cc. of oxygen are required for supplying the energy to saturate the air of superventilation. Adding the equivalents of the work of ventilation, of warming and saturating the air and of liberating carbon dioxide from solution it is seen that the total falls somewhat short of total superbasal dissipation of oxidative and anaerobic energy. A further reduction of the balance is conceivable as a result of expenditure of energy for chemical adjustments such as the secretion of the adrenal gland leading to facilitation of oxidations and also expenditure of energy for the repair of cellular damage done during the period of impaired oxidations.

The excess of energy dissipated (aerobic and anaerobic for the duration of the periods studied) was greatest during the period of lowered alveolar oxygen—approximately 19.2 per cent, 15.4 per cent and 10.5 per cent respectively of the basal consumption. For the periods of recovery it was 9.1 per cent, 5.7 per cent, and 6.3 per cent respectively. The average figures for the combined periods were 12.5 per cent, 10.0 per cent, and 7.6 per cent. Attention is called to the fact that in no case had the oxygen consumption returned to the basal level at the end of the observed recovery period

suggesting, that the energy equivalent of cellular repair to membranes, etc., may be a large factor in the final energy balance of perfectly restored tissue. Increased permeability of cell membranes which is known to occur as a result of impaired oxidations supports this view.

In the graphs and preceding description of results the formation and disappearance of lactic acid have been briefly considered in relation to oxygen consumption, carbon dioxide elimination, expiratory quotient, carbon dioxide capacity of the blood and total dissipation of energy. In the following table other relationships are recorded throwing light on the general problem of the significance of lactic acid as a source of food, of energy and a means of acid base control. It seemed desirable to learn of the distribution of lactic acid between blood plasma and tissue cells during normal basal metabolism and to compare this distribution with that at the end of a period of administration of low oxygen mixtures during which blood lactic acid is known to increase. Lactic acid determinations were made on whole blood, blood plasma, blood corpuscles, striated muscle and testicles by the method of Friedemann, Cotonio, and Shaffer (1927). An hour before taking blood and tissue samples symmetrical extensor muscles were dissected free of neighboring tissue in both hind legs to permit a subsequent rapid removal. The nerve and blood supplies were left intact and the skin was closed over the field to approximate normal conditions. Similar preparations were made for rapid removal of the testicles by freeing the testicles and pedicles from the surrounding tissue. When conditions of the animal were apparently uniform, as indicated by the steady head of blood pressure and uniform volume of ventilation, an arterial blood sample was drawn. Then two combination scissors and clamps were carefully slipped under the ends of one of the extensor muscles. At a signal, two operators simultaneously cut and clamped their respective ends of the muscle and the third operator dropped the intervening muscle in liquid air. With smooth technique, the muscle was dropped into the liquid air within one second after the signal. Shortly after the muscle sample was taken the testicle was removed. If sensory stimulation occurred as a result of excision, time was allowed for reëstablishment of more constant conditions, then low oxygen mixtures were administered. At the very end of this period, three similar samples of blood, muscle and testicle were taken which was immediately followed by readministration of room air.

The tissues were kept in liquid air during the experiment and then sectioned in the frozen condition with a microtome. The success of sectioning depends on a firm grip on the tissue and thorough freezing. An adjustable clamp was devised to hold the muscle and testicle and the whole procedure was carried out in a large refrigerator. Four to six samples of approximately 1 gram each were obtained from each muscle and testicle. By occasional cooling of the tissue and clamp in liquid air, easily manipulated

frozen sections were obtained which were dropped into weighted amounts of fixing solutions as they were cut. The temperature and thinness of the section and the temperature of the fixing solution would seem to offer ideal conditions for a minimum formation of lactic acid in the excised tissue as well as for establishment of complete equilibrium between tissue and fixing fluid.

In experiment 1 (see table 2) the basal values for whole blood, plasma, corpuscles, muscle and testicle were 16.5, 20.9, 13.4, 41.0, and 11.0 mgm. per 100 grams. At the end of the period of low oxygen these increased to 54.5, 75.3, 41.7, 55.0, and 19.0 mgm. respectively. The largest increase amounting to 54.4 mgm. occurred in the plasma. The corpuscular content increased 28.3 mgm., the muscle content increased 14.0 mgm., and the testicle content increased 8 mgm. Looking upon muscle as the probable

TABLE 2

Distribution of lactic acid before and after anoxemia

EXPERIMENT		BLOOD LACTIC ACID	PLASMA LACTIC ACID	CORPUSCLE LACTIC ACID	MUSCLE LACTIC ACID	TESTICLE LACTIC ACID
		mgm./100 cc.	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 grams*	*mgm./100 grams*
1	Basal	16.5	20.9	13.4	41	11
	After 6.09% O_2	54.5	75.3	41.7	55	19
	Increase	38	54.4	28.3	14	8
2	Basal	60			54	31
	Low O_2 (5.69%)	120			101	75
	Increase	60			47	44
3	Basal	17	16.2	13.7	30	12
	After 7.24% O_2	31.9	43.6	28.1	43	19
	Increase	14.9	27.4	14.4	13	7

primary source of lactic acid, it is of interest that the muscle increase should be lower than that of the plasma which may be looked upon merely as a vehicle of lactic acid. Obviously a significant redistribution of lactic acid has occurred.

Basal concentrations show a higher value for plasma than for corpuscles, the difference amounting to 7.5 mgm. per 100 cc. of blood. This is in agreement with the findings of Hill, Long and Lupton (1924); Gesell, Krueger, Gorham, and Bernthal (1930); and Eggleton and Evans (1930). A similar excess of plasma content over cell content (9.9 mgm. per 100 G.) is seen for the testicle. The muscle content is, however, decidedly higher than that of the plasma suggesting that lactic acid moves from the primary source of high concentration to the blood along a positive gradient. In a similar way, it might be inferred that lactic acid is diffusing into the

blood corpuscle and into the cells of the testicle along a positive gradient. The relatively low values of testicular content might conceivably be due to a slow diffusion of lactic acid into the cells and a relatively rapid consumption after it has entered. In the case of the corpuscles a relatively rapid consumption of lactic acid most probably does not occur. The distribution of lactic acid between plasma and corpuscles perhaps is, therefore, best explained by the Donnan membrane equilibrium.

That various tissues may remove lactic acid from the blood is now well established—striated muscle, Barr and Himwich (1923); cardiac muscle, Himwich, Koskoff, and Nahum (1930); brain, McGinty (1929); liver, Himwich, Koskoff, and Nahum (1930); McClure (1929); and Eggleton and Evans (1930). The experiments of McClure (1929), showing an increased difference in the hydrogen ion concentration between portal and hepatic blood following an increase in blood lactic acid produced by intravenous injection of sodium cyanide, indicate augmented removal of lactic acid from the blood stream. The liver thus tends, not only to continue a positive diffusion gradient for lactic acid from muscle to blood, but to con-

TABLE 3

Order of lactic acid concentration

EXPERIMENT	BEFORE OXYGEN LACK					AFTER OXYGEN LACK				
1	M	P	B	C	T	P	M	B	C	T
2	B	M			T	B	M			T
3	M	B	P	C	T	P	M	B	C	T

tribute base to the blood by conversion of lactic acid into glycogen. The liver, consequently, acts in a very important way to control the acid-base equilibrium of the body. A continuous removal of lactic acid from the blood by various organs and tissues might, therefore, influence the general distribution of lactic acid and account for the lower concentration of lactic acid in the arterial blood than in muscle as found in these experiments.

But since lactic acid appears to be a product of anerobic metabolism of all mammalian tissues it is reasonable to assume that with major anoxemia all tissues would be forming lactic acid faster than they are consuming it. The lowering of the arterial level below muscle level would then come to an end. McGinty's (1929) direct experiments on the brain show that during minor anoxemia the brain continues to absorb lactic acid from the arterial blood stream, but when impairment of oxidations increases, the brain, like muscle, contributes lactic acid to the blood. To what extent the contribution of lactic acid to the blood by the tissues in general and its removal by the liver counteract each other during varying degrees of impairment of oxidations is not known. It will be seen that lactic acid increases in

amount in the blood plasma with the progress of anoxemia and actually increases to a concentration considerably above that of muscle. This higher concentration, therefore, raises two significant questions. Is some tissue other than muscle contributing lactic acid to the blood stream at a greater rate than muscle and thereby raising the blood concentration, or has the failure of adequate removal of lactic acid from the blood by the liver and other tissues simply brought into evidence membrane activity leading to a demonstrable partition of lactic acid between the cell and surrounding fluids? Accepting the view that the hydrogen ion concentration of the interior of cells is greater than that of the environment, the Donnan membrane equilibrium calls for a lower value of lactic acid in the red blood corpuscle, muscle and testicle as compared with blood plasma. Assuming another possibility that the hydrogen ion concentration of the mammalian tissue cells decreases to a smaller extent than that of the blood plasma and tissue fluids with the progress of anoxemia (see below) the Donnan membrane equilibrium becomes of increased general importance in explaining the distribution of lactic acid during lowered alveolar oxygen.

In experiment 2 lactic acid also increased in the blood, muscle and testicle as a result of lack of oxygen. Separate determinations of plasma and corpuscular lactic acid content are missing, but since there were no other exceptions to the rule (eight experiments) and since it is a fact well established by other workers it is assumed that in this experiment, too, the plasma content was higher than the corpuscular content. Comparison of the concentration of whole blood with that of muscle during basal conditions shows the blood content to be higher than that of muscle. This difference increased with anoxemia. In experiment 3 the results are more comparable to those of experiment 1. During basal conditions the muscle content was higher than the plasma content and the corpuscle and testicle contents were lower. During anoxemia the plasma content increased most and just exceeded the muscle content.

By adding the energy liberated from the formation of lactic acid to the caloric equivalent of oxygen consumed, a so-called total dissipation of energy was obtained during the period of oxygen lack, and by subtracting the energy absorbed by reconversion of lactic acid to the precursor state from the caloric equivalent of oxygen consumed the total energy dissipated during the period of recovery was obtained. Since there is both an increased formation of lactic acid and an increased oxygen consumption during the period of lowered alveolar oxygen the increase in dissipation of energy is relatively larger than during the period of recovery where the absorption of energy represented by the lactic acid shift is subtracted from the oxidative energy.

The mechanisms controlling the dissipation of energy may be numerous, but it would appear that changing concentration of lactic acid could influ-

ence oxidations in two important ways. By increasing the hydrogen ion concentration of the tissues, lactic acid might tend to decrease oxidations and by increasing the amount of oxidizable material it might tend to augment oxidation. Since carbon dioxide is blown off in increased amounts during anoxemia while lactic acid is accumulating the acid effects of reduction in buffer base are counteracted. The synchronous accumulation of lactic acid and reduction in carbon dioxide, permitting oxidations to continue at a relatively high rate, then become a self corrective joint measure maintaining dissipation of energy at an adequate level. Just to what extent the blowing off of carbon dioxide opposes the acid effect of accumulation of fixed acid and reduction of buffer base cannot be accurately determined with the data at hand, but since the question is of importance in the control of respiration and ventilation we have attempted a tentative analysis. A refinement of procedure and conclusions must await later experiments.

For a rough approximation of the gross change in tissue acidity throughout the body as a whole, it was assumed that all the tissues have a similar metabolism, and are similarly affected by lack of oxygen, that the circulation is uniform throughout and that changes in the carbon dioxide pressure in the tissues are proportional to the changes in carbon dioxide pressure in the expired air. Obviously these simple conditions do not obtain. Nevertheless they are assumed, as a working basis, to conjecture the possible changes in hydrogen ion concentration of the tissues.

Employing Hasselbalch's equation and using for the respective values of the variables during basal conditions the subscript (B) and experimental conditions the subscript (E) we have

$$pH_B = K + \text{Log} \frac{(\text{Total } CO_2)_B - (\text{Free } CO_2)_B}{(\text{Free } CO_2)_B}$$

$$pH_E = K + \text{Log} \frac{(\text{Total } CO_2)_E - (\text{Free } CO_2)_E}{(\text{Free } CO_2)_E}$$

The change in pH is given by

$$pH = pH_B - pH_E = \text{Log} \frac{(\text{Total } CO_2)_B - (\text{Free } CO_2)_B}{(\text{Free } CO_2)_B} -$$

$$\text{Log} \frac{(\text{Total } CO_2)_E - (\text{Free } CO_2)_E}{(\text{Free } CO_2)_E}$$

Assuming an average basal total carbon dioxide content of the tissue of 24 cc. the corresponding value at another time may be obtained by subtracting the non-oxidative CO_2 which appears during the interval. The

partial pressure of carbon dioxide in the tissues during basal condition is assumed to be 50 mm. Hg.

With these values the changes in pH will be:

$$pH = pH_B - pH_E = \text{Log}\frac{24 - (0.0684 \times 50)}{(0.0684 \times 50)} - \text{Log}\frac{(24 - N) - (0.0684 \times 50)F}{(0.0684 \times 50)F}$$

where N is the average change in carbon dioxide content or the non-oxidative carbon dioxide eliminated between the two states and F is the ratio of the carbon dioxide in the expired air during rest to that during the administration of low oxygen. The changes in pH computed in this way are tabulated and presented in table 4. Negative values indicate a greater alkalinity of the tissues at the end of the period of oxygen lack. The computations (see table 4) therefore suggest that the alkaline effect of greater elimination of carbon dioxide more than compensates the acid effects of

TABLE 4

Calculated changes in tissue acidity

1	2	3	4	5	6
CO_2 EQUIVALENT OF INCREASE IN LACTIC ACID	NON-OXIDATIVE CARBON DIOXIDE	PARTIAL PRESSURE OF CARBON DIOXIDE IN EXPIRED AIR		pH OF TISSUES, CALCULATED	pH OF BLOOD, OBSERVED
		B	E		
cc./kgm.	*cc./kgm.*	*mm.* Hg	*mm.* Hg		
3.50	11.83	30.0	15.4	−0.00	−0.10
11.75	12.16	21.7	13.0	−0.10	−0.00
			18.4	−0.29	−0.05
3.25	7.93	34.2	14.0	+0.24	+0.21

accumulation of lactic acid in experiments 1 and 3 producing a resultant decreased hydrogen ion concentration of the tissues. In experiment 2, pH changes are given for gas sample 13 when the carbon dioxide pressure was 13 mm. Hg and for gas sample 14 when it was 18.4 mm. Hg. At this stage the animal was failing very rapidly, lactic acid was accumulating at a high rate, both blood pressure and ventilation were falling off. It is therefore highly probable that the computed increase in tissue hydrogen ion concentration above the normal is grossly correct. Note also that the pH of the blood was at the same time changing in a similar direction.

Though these experiments indicate some of the chemical processes which are at work supporting oxidations and the dissipation of energy they do not indicate specifically the chemical mechanisms controlling ventilation. To be sure, our calculated decrease in the gross hydrogen ion concentration of the animal might be interpreted as establishing a decrease in hydrogen ion concentration of all of the cells of the body including the respiratory neurones which control the respiratory act. But this by no means follows.

For the present all that can be said is that pulmonary ventilation is stimulated by some effect or several effects of lack of oxygen. The effects of lack of oxygen must be numerous— changes in concentration of the chemical constituents of the cell, changes in concentration of ions including the lactate, bicarbonate and hydrogen ions, changes in oxidation and reduction potentials, changes in the structural state of neuro-membranes including permeability, etc.

If increased ventilation from lowered alveolar oxygen is due to some effect other than increased hydrogen ion concentration, augmented ventilation might continue at a magnitude sufficient to turn the hydrogen ion concentration of the respiratory neurones to a subnormal level without necessarily entailing subnormal ventilation. On the other hand, if increased hydrogen ion concentration of the respiratory neurone is the stimulus, then ventilation must stop short of a point which will turn the neurones more alkaline than normal. If it did not, apnea or subnormal ventilation would result. Assuming an acid mechanism of control, it is necessary to conclude that the tissues as a whole were turned more alkaline than normal by virtue of an increase in the hydrogen ion concentration of the neurones controlling ventilation. Either view is compatible with the findings of our present experiments.

While the experiments showing rapid formation of lactic acid in the brain under anaerobic conditions (McGinty and Gesell, 1925) and reduced absorption of lactic acid from the blood or increased formation with graded impairment of oxidations (McGinty, 1929) and reduced carbon dioxide capacity with impaired oxidations support the possibility of an acid mechanism of control, they are not interpreted as establishing the fact. Neither are our present experiments suggesting that the brain as a whole may have turned less acid than normal during lowered alveolar oxygen interpreted to mean that the respiratory neurones have experienced the same directional change. We are inclined to believe that positive conclusions regarding the hydrogen ion concentration of the respiratory neurones during a response to lowered oxygen pressure are fraught with serious difficulty.

In this connection we are reminded of the recent report of Myerson, Loman, Edwards and Dill (1931) in which they state

> Our observations are more in line with those of Gesell and associates (1929). He has come to the conclusion that hydrogen ion concentration can no longer be considered the universal stimulus to respiration but that the metabolic processes in the center itself must be considered. As pointed out frequently by Henderson (1928) it is illogical to assign unique functions to a single variable such as $(H)^+$ when variations in it are necessarily associated with changes in concentration of other ions.
>
> Our results fit in very well with those of Cullen and associates (in press) who have shown that when patients with heart disease are exercised a large increase in pulmonary ventilation takes place with no increase in acidity of blood passing through the

brain. So far as we can judge the increase in pulmonary ventilation which occurs in anoxemia is associated with a normal oxygen supply to the brain and with more alkaline reaction than normal in blood leaving the brain.

While we wish to maintain our position that the control of ventilation is undoubtedly a multiplex phenomenon even to the extent that factors accessory to the hydrogen ion may under certain conditions be important enough to permit increased ventilation with subnormal hydrogen ion concentration of the respiratory neurones, we find difficulty in agreeing with the inference that the *respiratory neurones* do turn less acid during anoxemia. There are a number of points which need careful consideration in this connection to determine definitely whether an acid or alkaline effect occurs.

The mixed cerebral blood sample may not contain the outflow from the respiratory center, or if it does, the blood coming from the center may be so small in amount compared with the total cerebral flow that even a gross change in its composition would remain unregistered in the analysis of the total mixed sample. It is not at all inconceivable that the actual effective portion of the respiratory neurone arc responsive to chemical excitation may be only 0.001 to 0.0001 or even less of the total brain bulk.

The rate and nature of acid metabolism of the respiratory center cannot be assumed to be the same as that of the brain as a whole. Just as Haldi (1931) has shown that the rate and amount of lactic acid formed in isolated tissues under anaerobic conditions varies with the tissue so Haldi, Ward and Woo (1927) showed that the percentage content of lactic acid varies in different parts of the brain. More than twice as much lactic acid is formed in a unit weight of medulla tissue than in the tissue of the cerebral hemispheres.

The facilities for supply of oxygen and removal of acid must vary throughout the brain, for the distribution of capillaries is known to vary from one region to another (Craigie, 1926). Whether a cell or a block of tissue nourished by a given set of capillaries will turn more acid or less acid during lowered alveolar oxygen will depend upon the rate and nature of the acid metabolism, the pressures of oxygen, of carbon dioxide, and of lactic acid inside and outside of the cell, the distances of diffusion and the relative rates of diffusion of oxygen and carbon dioxide and lactic acid, and the buffering value of the tissue. If the requirements for oxygen are high and distances of diffusion are great, anaerobic metabolism with its consequent formation of lactic acid is likely to occur. A high lactic acid formation per unit of oxygen lack and a low rate of diffusion of lactic acid provides additional conditions for increased hydrogen ion concentration.

Increased functional activity of the respiratory neurones and consequent increased requirements for oxygen and removal of acids are other factors to be considered. Garrey (1920) has shown increased formation of acid in activated neurones. Fenn (1928) has shown the same for nerve fibers.

The problem of determining whether or not the respiratory neurones turn acid with lowered alveolar oxygen is obviously a difficult one. A direct experimental approach such as isolation or cannulation of a vein coming from the effective respiratory neurones seems for the present virtually impossible. New approaches must be made. One of us (Krueger, to be published) has attempted to interpret in terms of hydrogen ion concentration the conditions obtaining in the brain with respect to the requirements of oxygen and the facilities for diffusion of oxygen, carbon dioxide and lactic acid. Computations, to be sure, based on insufficient data confirm the suspicion that the hydrogen ion concentration of the larger brain cells or collection of brain cells without individual capillary supply may increase with lowered alveolar oxygen while the smaller cells suffer a decrease. We have already called attention to the computations of the present paper indicating that the body as a whole turns more alkaline with lowered alveolar oxygen.

In addition to these considerations the problem is involved from another important angle for it is no longer possible to consider the respiratory center as the only seat of chemical control.

The important experiments of Heymans (1931) demonstrated that the carotid sinus and the afferent vagus nerve endings are sensitive to lowered oxygen pressure, sodium cyanide and sodium sulphide. These results have been confirmed by Owen and Gesell (1931). While finding marked stimulation from local action of cyanide at the sinus Owen and Gesell were also able to demonstrate definite though smaller stimulation from arterial injection after denervation of the sinus and from flooding of the 4th ventricle. Painting of the carotid sinus and the floor of the fourth ventricle with weak sodium sulphide solution had marked and apparently equal stimulating effects. In agreement with Heymans, injections of acid and base were apparently equally effective whether these substances were injected central or peripheral to the sinus.

Of interest are the experiments from this laboratory of Glazer (1929), Winkler (1930), and Gay (1931) which demonstrated changes in excitability of various portions of the reflex arc under several conditions modifying pulmonary ventilation. Winkler showed fluctuations in the reflex response of the tibialis anticus running parallel to fluctuations in ventilation accompanying hemorrhage and reinjection. Gay (1931) showed a similar parallelism with administration of sodium cyanide, sodium sulphide, and gaseous mixtures low in oxygen. On the other hand, administration of carbon dioxide was found to lessen the reflex response. These workers suggested accessory chemical control, peripheral to that in the so-called respiratory center, operating on the peripheral sensory and motor fibers, on the cord and on muscle directly.

Recent experiments, therefore, indicate both central and peripheral

regulative mechanisms seemingly of varying kind and significance in the control of pulmonary ventilation. Whether the less certain effects of central action of cyanide as compared with sinus stimulation are attributable to a resultant of excitation at one point in the respiratory neurone chain and depression at another remains for further study. Why lowered alveolar oxygen augments spinal reflexes and increased alveolar carbon dioxide depresses them also requires further consideration. Regardless of possible interpretations, it is now as essential to determine how chemical changes excite peripherally as it is to determine how they act on the so-called respiratory center. This obviously will require further chemical studies but along with these must go intensive and detailed study of the respiratory nerve impulse at various stations in the complex respiratory reflex arc.

Still ignorant of the fundamentals involved in the control of the respiratory nerve impulses our views regarding the chemical changes which are responsible for the control of ventilation must remain indefinite. While our present data do not seem to be opposed to a significant acid mechanism of control under various conditions it is reminded that (Gesell, 1929)

> the theory in no way proposes that the hydrogen ion is the one and only regulator of respiration for obviously the hydrogen ions are operating on a highly complex and relatively unstable mechanism about which we know very little. For example, the state of oxidation of the neurone membrane may be a contributing factor of control. The position that tissue acidity is the only factor controlling ventilation is thus untenable. To quote from my last review (1925) "It should be recalled that the regulation of respiration in its ultimate analysis may be found to be an electrical phenomenon occurring with the aid of a surface membrane, and changes in composition of the fluids on both sides of that membrane. Whether acid is the only agent which can produce the necessary electrical disturbance involved in the control of respiratory nerve impulses is highly improbable; on the other hand, it may be the agent most employed in the body."

SUMMARY AND CONCLUSIONS

In a preceding series of papers numerous factors relating to the control of respiration and ventilation were followed simultaneously on individual animals in which ventilation was artificially administered and maintained at a uniform volume.

In the present series of experiments a similar study was made with pulmonary ventilation under the control of the respiratory mechanisms of the body.

We, therefore, compare in unusual detail the essential findings in both series in order to accentuate the effects of controlled ventilation and the conditions under which augmented ventilation occurs.

During uniform ventilation, administration of gaseous mixtures low in oxygen invariably decreased oxygen consumption and readministration of

room air temporarily increased oxygen consumption considerably above the normal level. The excess oxygen consumption as a rule was less than the preceding shortage.

With ventilation under normal control oxygen consumption increased, both during the period of lowered alveolar oxygen and during the subsequent period of recovery. The increase in oxygen consumption during lowered alveolar oxygen was temporary and relatively small.

Carbon dioxide elimination during constant ventilation changed much less than the coincident fluctuations in oxygen consumption. In most experiments carbon dioxide elimination decreased, in some experiments it remained virtually unchanged and in others it increased slightly above normal. Readministration of room air almost invariably increased elimination of carbon dioxide above the normal level. In one experiment in which carbon dioxide elimination was greater than normal during lowered alveolar oxygen pressure the primary effect of readministration of room air was a reduction in carbon dioxide elimination.

In contrast to these results, during normally controlled ventilation carbon dioxide elimination increased enormously during the period of lowered alveolar oxygen and decreased below normal with subsequent administration of room air. The increase in elimination during lowered alveolar oxygen was greater than the decrease below normal during recovery. The increased elimination was greatest during the early part of lowered alveolar oxygen and the decreased elimination was greatest during the early part of the period of recovery.

These alterations in gaseous exchange resulted in fluctuations in the expiratory quotient—an increase during the period of administration of gaseous mixtures low in oxygen and a decrease below normal following the readministration of room air. This held for both series of experiments—uniform and variable ventilation. The greatest increase in the expiratory quotient obtained during the initial period of oxygen lack and the greatest decrease during the early part of the recovery. The respective increases and decreases in the expiratory quotients were greater in the experiments in which ventilation was normally adjusted.

Lactic acid accumulated in the arterial blood during the period of lowered alveolar oxygen and decreased or showed a tendency toward a decrease with readministration of room air. This held for both series of experiments. During lowered alveolar oxygen the increase was greater in the experiments with uniform artificial ventilation. The decrease during recovery was more pronounced in the experiments in which ventilation was under normal control. In two experiments (nos. 2 and 3) with normally adjusted ventilation, the increase in lactic acid during lowered alveolar oxygen temporarily gave way to a short period of decreasing lactic acid content.

Administration of gaseous mixtures low in oxygen during constant ven-

tilation was accompanied by a decrease in the hydrogen ion concentration of arterial blood as measured with the quinhydrone electrode. This occurred despite the accumulation of lactic acid in the blood and the reduction of carbon dioxide elimination from the lungs. Readministration of room air produced a temporary increase in the hydrogen ion concentration followed by a return towards the normal level. The same directional changes occurred during normally controlled ventilation, but the decrease in hydrogen ion concentration during lowered alveolar oxygen was greater and the increase above normal with readministration of room air was less. Since the decrease in hydrogen ion concentration of the arterial blood during lowered alveolar oxygen and uniform ventilation was associated with an increase in lactic acid and a decreased elimination of carbon dioxide, it was attributed primarily to the reduced state of the hemoglobin. The increase in hydrogen ion concentration above normal occurring with readministration of room air was attributed to the lowered buffer base of the blood from the accumulation of fixed acid and to the increased carbon dioxide pressure resulting from suddenly augmented oxidations. The greater decrease in hydrogen ion concentration during lowered alveolar oxygen with normally controlled ventilation was attributed to the freer blowing off of carbon dioxide from the blood and tissues. The smaller increase in hydrogen ion concentration with readministration of room air was attributed to the greater desaturation of the blood and tissues at the moment augmented formation of carbon dioxide was reëstablished.

The increase in the expiratory quotient above normal during lowered alveolar oxygen and uniform pulmonary ventilation was attributed to the formation of lactic acid and its reaction with bicarbonate, and to increased volume flow of blood. To this should be added the greater carrying power of the less perfectly oxidized blood assisting a freer elimination of carbon dioxide (Henderson, 1928). The greater increase in the expiratory quotient during normally controlled ventilation was attributed to the freer elimination of carbon dioxide resulting from greater ventilation. The decrease in the expiratory quotient with readministration of room air during uniform ventilation was attributed to a liberation of free base on reversion of the lactic acid cycle and to a reduction in volume flow of blood. The greater decrease in the expiratory quotient during normally controlled ventilation when room air is readministered was attributed to the sudden reduction in ventilation, to the desaturated condition of the tissues and blood and possibly to a more perfect reversal of the lactic acid cycle leading to a freer liberation of base. The changes in the expiratory quotient are thus attributed primarily to fluctuations in non-oxidative carbon dioxide—eliminated and retained.

In the present experiments, in which ventilation was normally controlled, the distribution of lactic acid between the blood plasma and the red blood

cell, striated muscle cell and the cells of the testicle was determined. In two experiments out of three, during administration of room air, the lactic acid content was highest in muscle, lowest in the testicle, and second lowest in the red blood cell. Plasma and blood concentrations were variable. During lack of oxygen there was a differential increase in lactic acid. Beginning with the highest the new order of concentration was plasma, muscle, whole blood, red blood cell, and testicle.

It was tentatively suggested that muscle is the primary source of blood lactic acid; that blood lactic acid may pass into various tissues where it in turn may be consumed or deposited as a lactic acid precursor; that lactic acid may pass into the blood stream along a positive or negative diffusion gradient; that the lower concentration of lactic acid in arterial blood plasma as compared with striated muscle under basal conditions may be due to the removal of lactic acid from the blood stream by the liver, heart, brain and other organs; that a failure to remove lactic acid from the blood stream during severe oxygen lack results in its accumulation in the blood and tissues. This accumulation in the blood, it is suggested, permits a demonstrable distribution of lactic acid agreeing with the Donnan membrane equilibrium.

Assuming equal distribution of lactic acid between the blood (whole blood was used) and the tissues, it was concluded that in the uniform ventilation experiments approximately 40 per cent of the lactic acid which was liberated combined with bicarbonate to form carbonic acid. Since the present experiments indicate that lactic acid accumulates in the blood more than in the tissues the amount reacting with bicarbonate may therefore have been somewhat higher.

By adding the caloric equivalents of oxygen consumed to the energy shift accompanying the lactic acid shift, total energy production was computed. With uniform ventilation energy production fell, as a rule, during the period of oxygen lack and invariably rose above the normal level on readministration of room air. When the rate of lactic acid production per unit of oxygen shortage was high the total energy production increased above normal during the period of oxygen lack.

In the experiments in which ventilation was under normal control the total dissipation of energy increased both during the period of administration of gaseous mixtures low in oxygen and during the subsequent period of recovery. The increase in energy dissipation during lowered alveolar oxygen (caloric equivalent of excess oxidations plus energy liberated with the formation of lactic acid) was greater than the increase in energy dissipated during an equal period of recovery (caloric equivalent of excessive oxidations minus the energy absorbed as a result of transformation of lactic acid to its precursor state).

It was concluded that excess energy is required for adjusting the animal to

lowered oxygen pressures and for recovery from the effects of lowered alveolar oxygen. It is suggested that 1, the work of ventilation involved in moving the extra air; 2, the warming of the extra air; 3, the saturation of the extra air with water vapor; 4, the liberation of carbon dioxide from the blood into the alveolar air; 5, the increased cardiac output; 6, the chemical adjustments for augmenting oxidations, and 7, the repair of cellular damage from impaired oxidations may be the primary energy consuming processes. The energy consumed was computed for processes 1, 2, 3 and 4. Of these the saturation of the superbasal air with water vapor represented the greatest expenditure of energy. The sum of the computed energy for processes 1, 2, 3 and 4 was found to be less than the superbasal dissipation of energy for the combined periods of lowered alveolar oxygen and recovery indicating that the repair of cellular damage from acute exposure to lowered oxygen pressures may be a costly expenditure of energy.

Assuming a uniform aerobic and anaerobic metabolism and volume flow of blood throughout the body an approximate computation indicating a decrease in the gross hydrogen ion concentration of the tissues is presented. According to these computations the decrease in hydrogen ion concentration is a result of a lowering of carbon dioxide pressure which more than compensates for the reduction of bicarbonate base by the accumulation of fixed acid.

It is suggested that oxidations are supported during lowered alveolar oxygen by an accumulation of oxidizable material such as lactic acid and by a general reduction in hydrogen ion concentration of the tissues.

Assuming an acid mechanism of control it is necessary to conclude that the tissues as a whole were turned more alkaline than normal during lowered alveolar oxygen by virtue of an increase in the hydrogen ion concentration of some portion of the respiratory neurone chain controlling ventilation. It is pointed out however that the present experiments do not indicate which of several chemical changes resulting from lowered alveolar oxygen are responsible for augmented ventilation.

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A COMPARISON OF THE RESPONSE OF THE ANESTHETIZED DOG TO INTRAVENOUS ADMINISTRATION OF SODIUM CYANIDE DURING UNIFORM ARTIFICIAL VENTILATION AND DURING NORMALLY CONTROLLED VENTILATION WITH ADDITIONAL OBSERVATIONS ON THE EFFECTS OF METHYLENE BLUE

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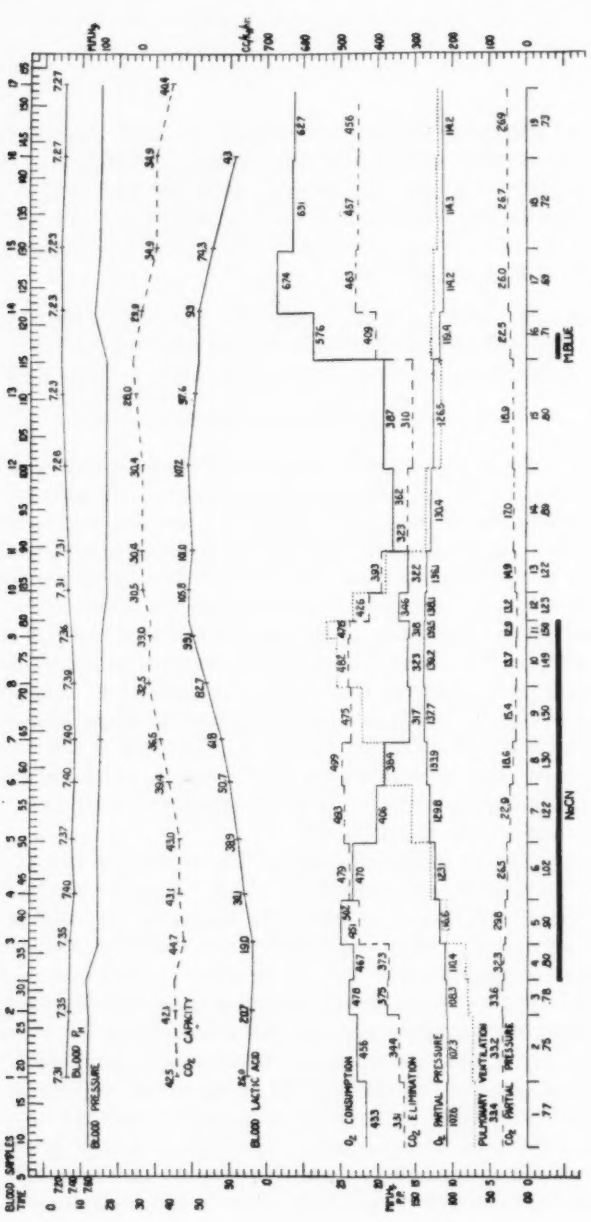
In this series of experiments oxidations were impaired by intravenous injection of 0.004 M. solution of sodium cyanide in isotonic salt solution. Cyanide was injected at a rate sufficient to increase ventilation and decrease oxidations just short of depression of the respiratory and circulatory systems. Continuous injection was accomplished by oil displacement of a cyanide solution contained in a 500 cc. burette sealed above. The oil was delivered to the bottom of the burette and at a pressure of approximately 400 mm. Hg. This relatively high pressure insures a constant rate of injection by minimizing the effects of variations of venous pressure. Allowing oil to rise in drops from an upward pointing dropper permits observation and control of the rate of displacement of the cyanide solution. Due to the accumulative action of cyanide, which not infrequently required a change in rate of injection, irregularity of pulmonary ventilation was occasionally unavoidable.

Four experiments were performed. The results of two of these are presented in graphic form in figures 1 and 2. Experiments 1, 3 and 4 are characterised by even and progressively increasing response to injection. In experiment 2 the effects of injection are more pronounced and the early vigorous response gives way to depression. This in turn is followed by partial recovery at the close of injection as in experiments 1, 3 and 4.

In experiment 1 sodium cyanide was administered for a period of 48 minutes. There was a continuous increase in ventilation from about 7000 cc. per kgm. hour to about 27000 cc. up to the very end of injection. This was accompanied by an increase in oxygen pressure of the expired air from 108.3 mm. to 139.5 mm. The carbon dioxide pressure of the expired air fell coincidentally from 33.6 mm. to 12.9 mm. Oxygen consumption fell from 478 cc. per kgm. hour to 318 cc. and carbon dioxide


BLOOD SAMPLES
TYPE S
BLOOD P_H
BLOOD PRESSURE
CO_2 CAPACITY
BLOOD LACTIC ACID
O_2 CONSUMPTION
CO_2 ELIMINATION
O_2 PARTIAL PRESSURE
PULMONARY VENTILATION
CO_2 PARTIAL PRESSURE
NICN
BLUE

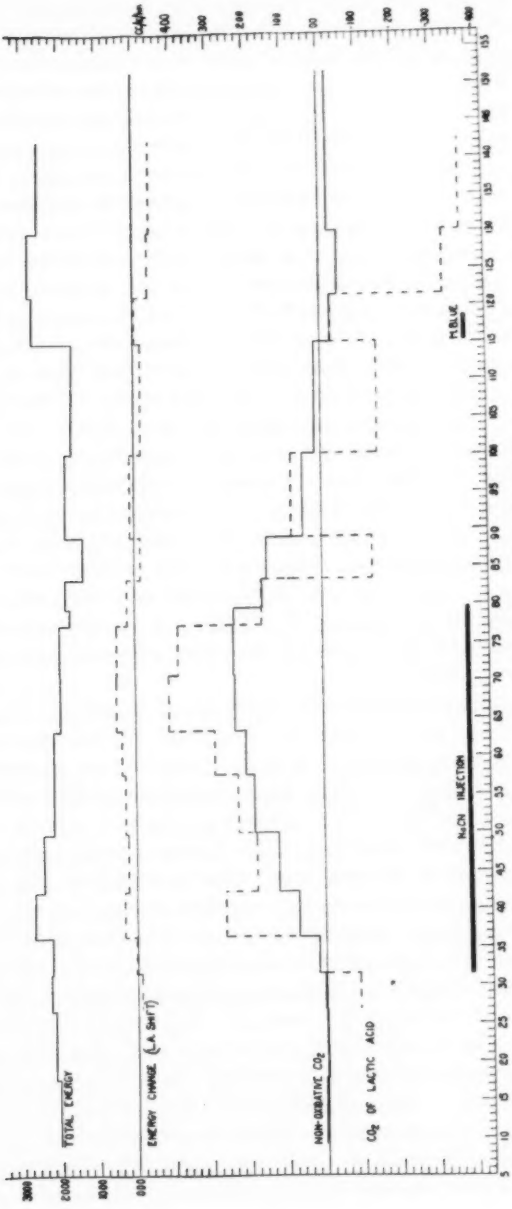


Fig. 1. Experiment 1, June 19, 1930

elimination increased from 375 cc. per kgm. hour to 478 cc. The expiratory quotient rose from 0.78 to 1.50. The blood lactic acid increased from 19.0 mgm. per 100 cc. to 99.1 mgm. This along with the increased ventilation, readily accounts for the increased expiratory quotient. The decrease in blood carbon dioxide was relatively small compared with the increase in blood lactic acid. The hydrogen ion concentration of the blood decreased from pH 7.35 to 7.40, but increased again towards the close of injection to pH 7.36 despite the progressively augmenting ventilation. This increase in hydrogen ion concentration is most probably associated with the rapidly increasing blood lactic acid concentration.

Recovery processes set in immediately at the close of injection and conditions progressively approached those of the preinjection period. Normal recovery processes were later interrupted by intravenous injection of methylene blue. Just prior to methylene blue administration pulmonary ventilation had fallen to approximately 11,500 cc. per kgm. hour. Oxygen consumption had increased from 318 cc. per kgm. hour to 387 cc., the partial pressure of expired oxygen falling in the meantime from 139.5 mm. to 126.5 mm. Carbon dioxide elimination had fallen from 476 cc. per kgm. hour to 310 cc. accompanied by an increase in the partial pressure of expired carbon dioxide from 12.9 mm. to 18.9 mm. The expiratory quotient had fallen from 1.50 to 0.80, which was associated with only a small drop in blood lactic acid from 105.8 mgm. per 100 cc. of blood to 97.6 mgm. The acidity of the blood increased progressively from pH 7.36 to 7.23 which is 0.08 pH more acid than that of the preinjection period.

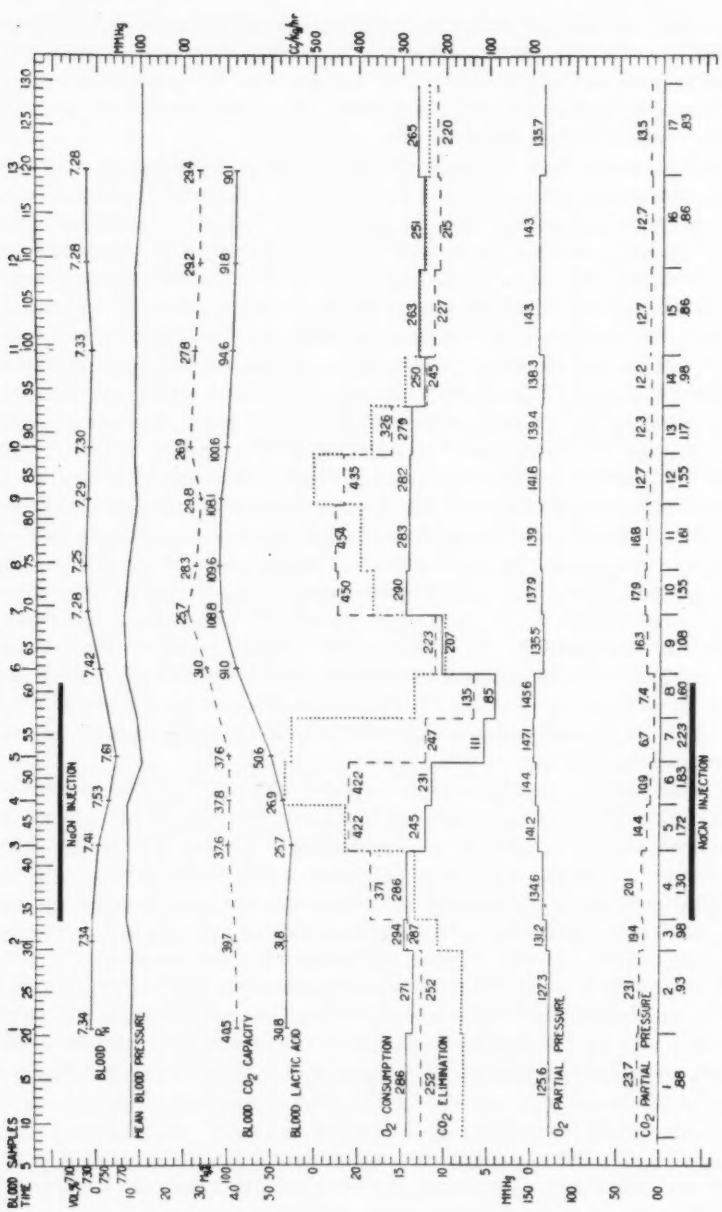
At gas sample 16, during the injection of methylene blue, striking changes occurred, all of which are suggestive of suddenly augmented recovery. Oxygen consumption increased from 387 cc. per kgm. hour to 674 cc. per kgm. hour. Carbon dioxide elimination increased from 310 to 463 cc. per kgm. hour. The partial pressure of expired oxygen decreased from 126.5 to 114.2 mm. Hg. Carbon dioxide partial pressure increased from 18.9 to 26.9 mm. Hg. The blood lactic acid content fell rapidly and the blood carbon dioxide capacity increased almost to normal values. The expiratory quotient fell to, and remained at, distinctly subnormal values. The hydrogen ion concentration of the blood returned almost to normal despite the increasing pressure of carbon dioxide in the expired air.

Special attention is called to the relatively small change in pulmonary ventilation, virtually none, accompanying a relatively large increase in gaseous metabolism. These results agree with those recently described by Eddy (1931) who showed that the effects of methylene blue on pulmonary ventilation vary, depending upon the prevailing conditions. If injected under normal conditions, an increase in pulmonary ventilation

invariably occurs, but if injected during hyperpnea, elicited by intravenous injection of cyanide, pulmonary ventilation may be depressed. The conclusion from such results is that the effects of increased oxidations on pulmonary ventilation vary, depending upon the conditions obtaining when oxidations are changed.

Experiment 2 shows striking changes during administration of cyanide and during recovery. During the first part of cyanide administration the animal reacted vigorously to impaired oxidations; ventilation, mean blood pressure and carbon dioxide elimination increased. Then the condition of the animal suddenly changed. The mean blood pressure fell, pulmonary ventilation decreased, oxygen consumption fell off rapidly, carbon dioxide elimination diminished, lactic acid accumulated at a faster rate and the hydrogen ion concentration of the arterial blood increased. During this stage the decrease in carbon dioxide capacity of the blood and the increase in lactic acid content of the blood ran more parallel than during the early period of administration. At the end of cyanide injection, there was a rapid return of hyperventilation accompanied by an increased consumption of oxygen, an increased elimination of carbon dioxide, a reduction in blood lactic acid, a fall in the expiratory quotient and a reduction in the hydrogen ion concentration of the blood. The temporarily increased ventilation occurring during the period of recovery stands in contrast to the decrease in ventilation occurring at the close of injection in experiments 1, 3 and 4. It is probably due to the fact that an acute depression occurred during the administration of cyanide and that during gas samples 10, 11, 12 and 13 the animal is passing from a condition of depression to one of recovery, permitting the cyanide to evoke a second stimulation.

On the whole, experiments 1, 3 and 4 showed that the greater the ventilation the lower was the carbon dioxide pressure of the expired air. Experiment 2 is an exception to these findings. During gas samples 7 and 8 where ventilation was falling off there was a decrease in the carbon dioxide pressure of the expired air and during the later samples in which ventilation was augmented, with the exception of gas sample 9, the carbon dioxide pressure of the expired air increased. These contrary findings may be related to the failure of the circulation to carry carbon dioxide to the lungs during gas samples 7 and 8 when the blood pressure was falling rapidly and to the improved transport of accumulated carbon dioxide when mean blood pressure and volume flow of blood was again increased during gas samples 9, 10, 11, and 12. The dissipation of energy, aerobic and anaerobic, is analyzed in the preceding paper. See figures 1 and 2 and table 1. When oxidations were impaired by the administration of gaseous mixtures low in oxygen it will be recalled that the total oxygen consumption was slightly increased. In the present experiments, on the


BLOOD SAMPLES
TIME
VOL%
MEAN BLOOD PRESSURE
NaCN INJECTION
BLOOD O_2
BLOOD CO_2 CAPACITY
BLOOD LACTIC ACID
O_2 CONSUMPTION
CO_2 ELIMINATION
O_2 PARTIAL PRESSURE
CO_2 PARTIAL PRESSURE
NaCN INJECTION
MMHg
cc/kg/hr

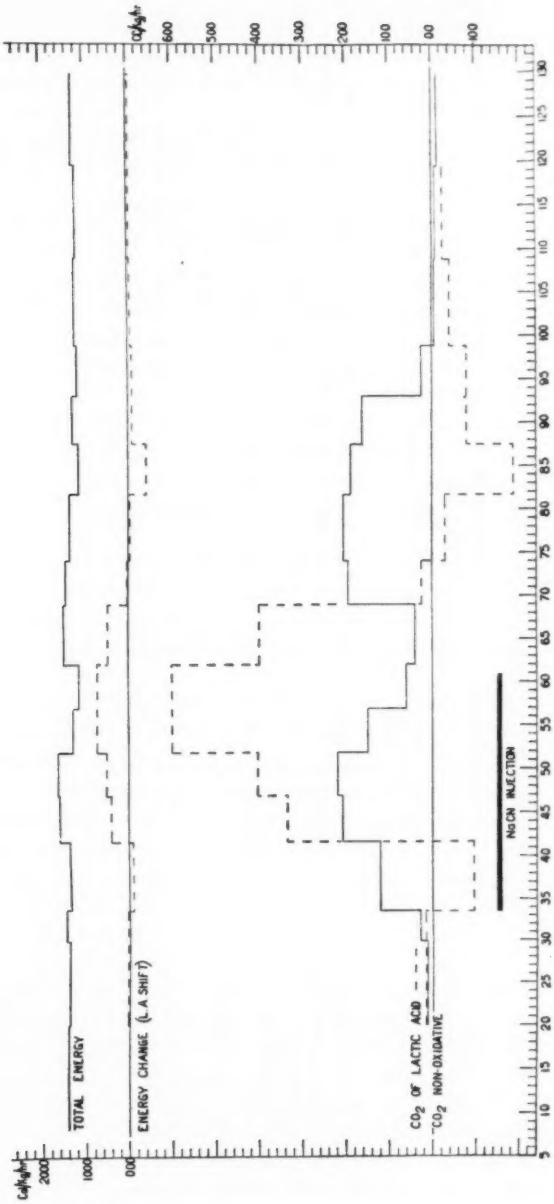


Fig. 2. Experiment 2, August 13, 1930

TABLE 1

Energy relationships

	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3			EXPERIMENT 4		
	NaCN G. S. 4-11	Recovery G. S. 12-15	NaCN and recovery G. S. 4-15	NaCN G. S. 4-8	Recovery G. S. 9-17	NaCN and recovery G. S. 4-17	NaCN G. S. 4-11	Recovery G. S. 12-16	NaCN and recovery G. S. 4-16	NaCN G. S. 4-10	Recovery G. S. 11-14	NaCN and recovery G. S. 4-14
1. Basal O_2 consumption	358.2	263.4	621.6	135.2	323.0	458.2	260.3	194.7	455.0	213.8	202.4	416.2
2. Observed O_2 consumption	324.1	215.8	539.9	95.3	296.1	391.4	217.1	145.5	362.6	188.7	196.6	385.3
3. Oxygen balance	−34.1	−47.6	−81.7	−39.9	−26.9	−66.8	−43.2	−49.2	−92.4	−25.1	−5.8	−30.9
4. Anaerobic energy	+53.7	−2.3	+51.4	+35.5	+1.0	+36.5	+41.0	−10.8	+30.2	+38.1	−8.0	+30.1
5. (3) + (4)	+19.6	−49.9	−30.3	−4.4	−25.9	−30.3	−2.2	−60.0	−62.2	+13.0	−13.8	−0.8
6. O_2 work (ventilation)	−0.2	−0.1	−0.3	−0.2	−0.2	−0.4	−0.4	−0.2	−0.6	−0.3	−0.1	−0.4
7. O_2 warming air	−6.2	−3.4	−9.6	−4.5	−6.5	−11.0	−7.2	−4.3	−11.5	−5.6	−2.5	−8.1
8. O_2 saturating air	−24.5	−13.7	−38.2	−17.8	−25.9	−43.7	−28.6	−16.9	−45.5	−22.3	−9.7	−32.0
9. O_2 CO_2 liberation	−13.4	−3.8	−17.2	−7.1	−6.2	−13.3	−12.6	−7.0	−19.6	−10.0	+0.3	−9.7
10. (6) + (7) + (8) + (9)	−44.3	−21.0	−65.3	−29.6	−38.8	−68.4	−48.8	−28.4	−77.2	−38.2	−12.0	−50.2
11. (5) + (10)	−24.7	−70.9	−95.6	−34.0	−64.7	−98.7	−51.0	−88.4	−139.4	−25.2	−25.8	−51.0

All values are given in cubic centimeters of oxygen per kilogram.

contrary, oxygen consumption was definitely reduced (see figs. 1 and 2, and items 1 and 2 in column 1 of each experiment in table 1). The oxygen balance is given in line 3. The anaerobic energy liberated in terms of cubic centimeters of oxygen is given in line 4. Line 5 (the sum of the oxygen balance and the anaerobic energy) indicates a deficit of energy below an anticipated basal rate equivalent to 2.2 and 4.4 cc. in experiments 3 and 2. In experiments 4 and 1 an excess above an anticipated basal rate amounting to 13.0 to 19.6 cc. occurred. These results differ from those of the low oxygen experiments in which the energy liberated during anoxemia was never less than the expected basal rate. The dissipation of energy during the recovery period differs still more from that of the lowered alveolar oxygen experiments in which oxidations were always markedly increased over the expected basal rate. In the cyanide experiments, oxidations during the period of recovery were always less than the expected basal rate as seen by a comparison of items 1 and 2 in column 2 of each experiment. Since lactic acid also decreased during this period the sum total of energy dissipated was less than the anticipated basal rate. The sum total of dissipated energy for the combined periods of administration and recovery (see item 5, column 3 of each experiment) was in every instance less than the anticipated basal rate. Adding this deficit to the energy required for the extra pulmonary ventilation, the warming and the saturating of the extra air with water vapor, and the energy of liberation of the extra carbon dioxide from the blood into the alveolar air gives the total computed negative energy balance. In experiments 1, 2, 3 and 4 there was a deficit of energy in oxygen equivalents amounting to 95.6 cc., 98.7 cc., 139.4 cc., and 51.0 cc. per kgm. In the three experiments in which gaseous mixtures low in oxygen were administered the same method of computations gave a positive balance of energy. A comparison of the results of the low oxygen and cyanide experiments would thus seem to indicate that impairment of oxidation by interference with the oxidative mechanism is more harmful than interference with the supply of oxygen. Whether this is due to a greater tissue acidity resulting from impaired transport of carbon dioxide by a relatively highly oxygenated blood, or to some other cause, is difficult to say.

In the preceding experiments the formation of lactic acid and its distribution between blood plasma and the corpuscles, striated muscle and testicles were discussed with the aid of tabulated determinations. In the cyanide experiments determination of the lactic acid content of blood and tissues were also made. They appear in table 2 and the order of concentration is summarized in table 3. In experiment 3 determinations were not made on the content of lactic acid in plasma and in the corpuscle, but only in whole blood, muscle and testicle. In general the highest

concentration obtained in muscle, the lowest in the testicle and the intermediate concentration in plasma, blood and corpuscles during basal metabolic conditions. In three experiments testicular content was lowest and in one the corpuscles had a slightly smaller content.

During the administration of cyanide there was a general increase in lactic acid content for blood and tissues but not an equal increase as is

TABLE 2

Distribution of lactic acid before and after intravenous injection of sodium cyanide

EXPERIMENT		BLOOD LACTIC ACID	PLASMA LACTIC ACID	CORPUSCLE LACTIC ACID	MUSCLE LACTIC ACID	TESTICLE LACTIC ACID
		mgm./100 cc.	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 grams*	*mgm./100 grams*
1	Basal	20.7	16.7	22	34	12
	After NaCN	99.1	111	75.8	62	60
	Increase	78.4	94.3	53.8	28	48
2	Basal	31.8	45.1	26.5	38	20
	After NaCN	91	138.2	73.3	91	68
	Increase	59.2	93.1	46.8	53	44
3	Basal	25.5			30	13
	After NaCN	86			75	50
	Increase	60.5			45	37
4	Basal	25.6	37.5	20.8	41	21
	After NaCN	84.4	98.6	61.6	68	57
	Increase	58.8	61.6	40.8	27	36

TABLE 3

Order of lactic acid concentration

EXPERIMENT	BEFORE INTRAVENOUS INJECTION OF SODIUM CYANIDE					AFTER INTRAVENOUS INJECTION OF SODIUM CYANIDE				
1	M	C	B	P	T	P	B	C	M	T
2	P	M	B	C	T	P	B	M	C	T
3	M		B		T		B	M		T
4	M	P	B	T	C	P	B	M	C	T

shown in the lower half of table 3. In all of the experiments in which plasma determinations were made, plasma concentration is now seen to be the highest. Due to the large increase in plasma lactic acid, blood concentration is second in order, muscle concentration has fallen from highest to third highest concentration, corpuscular concentration is fourth highest and testicular concentration remains the lowest. The results are very similar to the experiments on lowered alveolar oxygen. The mecha-

nisms leading to a basal distribution and effecting an altered distribution during impaired oxidations are probably the same and have been discussed in the preceding paper.

SUMMARY AND CONCLUSIONS

In a preceding series of experiments effects of intravenous injection of sodium cyanide were studied during administration of uniform ventilation with room air. In the present series of experiments the effects of injection were studied with pulmonary ventilation under physiological control.

A 0.004 M. sodium cyanide solution was injected 25 to 48 minutes at a rate sufficient to produce marked augmentation of pulmonary ventilation.

The results obtained are compared with those of cyanide injection and uniform ventilation and with those of lowered alveolar oxygen with uniform ventilation and with physiologically controlled ventilation.

In both series of cyanide experiments oxygen consumption was greatly decreased during the period of injection. These results are similar to the reduction of oxygen consumption accompanying the administration of gaseous mixtures low in oxygen during uniform ventilation but differ from the effects of lowered alveolar oxygen when ventilation is under normal control.

Hyper-oxygen consumption, common to recovery after oxygen lack, was missing during recovery from cyanide injection. Oxygen consumption of the recovery period was below the preceding basal rate.

Carbon dioxide elimination was decreased by cyanide injection during uniform artificial ventilation, but increased during physiologically controlled ventilation.

During recovery, with uniform ventilation, carbon dioxide elimination increased towards normal with a tendency to overshoot. With ventilation under physiological control carbon dioxide elimination decreased towards normal with a tendency to undershoot the basal rate.

The reduction in carbon dioxide elimination from cyanide injection during uniform ventilation was attributed to lowered oxidations and to impaired transport of carbon dioxide from the tissues to the lungs resulting from a permanently high oxygenation of the blood. The increased elimination of carbon dioxide during cyanide injection and physiological control of ventilation was attributed to the washing out of preformed carbon dioxide by augmented ventilation.

The increase in carbon dioxide elimination and tendency toward overshooting during recovery from cyanide under uniform ventilation was attributed to the increase in oxidations and improved transport of hyperaccumulated carbon dioxide in the tissues. The reduction in carbon dioxide elimination during recovery with physiologically controlled ventilation was attributed to the reduction in ventilation, to the relatively

desaturated condition of the tissues and blood, and to a more complete reversal of the lactic acid cycle and liberation of free base.

The expiratory quotient during cyanide injection and uniform ventilation was slightly increased, during physiologically controlled ventilation it was markedly increased.

The increase in the expiratory quotient during cyanide injection and uniform ventilation is attributed to the formation of lactic acid.

The relatively small increase in the expiratory quotient compared with that of lowered alveolar oxygen and uniform ventilation is tentatively attributed to an impairment in transport of carbon dioxide associated with a permanently high oxygenation of the blood. This inefficient transport is in contrast to an augmented carrying power, the result of a permanent low oxygenation of blood, obtaining with lowered alveolar oxygen.

The large increase in the expiratory quotient during cyanide injection and physiologically controlled ventilation is attributed to the augmented ventilation.

During recovery from cyanide injection during uniform ventilation, the expiratory quotient diminished, but not to subnormal levels as was the case in lowered alveolar oxygen during uniform ventilation. This relatively high recovery expiratory quotient is tentatively attributed to the combined effects of a greater accumulation of carbon dioxide in the tissues and to a more incomplete recovery of the oxidative mechanism of the cell with consequent failure of removal of lactic acid.

During recovery, under physiologically controlled ventilation, the expiratory quotient fell slightly below normal. This greater reduction in the recovery expiratory quotient, as compared with that of uniform ventilation, is attributed to a lower carbon dioxide content of the tissues at the time of returning oxidations and to a more complete recovery of the tissues permitting a more perfect reversal of the lactic acid cycle with liberation of free base. The relatively small reduction in the expiratory quotient as compared with the lowered alveolar oxygen experiments is again attributed to greater content of carbon dioxide in the tissues and a less perfect recovery of cellular functions.

Blood lactic-acid increased during cyanide injection in both series of experiments and decreased during recovery. The recovery decrease was greater in the experiments in which ventilation was physiologically controlled. Since in neither series were the animals suffering from lack of oxygenation of the blood, it is concluded that the better recovery in the experiments with physiologically controlled ventilation may be attributable to the greater elimination of carbon dioxide.

The relative order of concentration of lactic acid before injection of cyanide (muscle, plasma, whole blood, corpuscle, and testicle) was changed

at the end of injection to plasma, whole blood, muscle, corpuscles and testicles. These results are similar to those produced by lowered alveolar oxygen and are tentatively interpreted in the same way.

The carbon dioxide capacity of the blood was decreased during cyanide administration and increased during recovery. The changes were smaller than the corresponding changes in blood lactic acid. A tendency towards a slight and temporary increase in carbon dioxide capacity during the early part of cyanide injection was noted. The results agree in general with those of cyanide injection and uniform ventilation and of lowered alveolar oxygen.

The hydrogen ion concentration of the blood decreased during cyanide injection and increased towards normal during recovery with a tendency towards slight temporary overshooting. With uniform ventilation there was an increased hydrogen ion concentration during injection followed by a tendency towards recovery. Since cyanide injection and uniform ventilation were associated with a decreased alveolar carbon dioxide pressure, the increase in hydrogen ion concentration was attributed to an increased lactic acid content and an increased oxygenation of the blood. Since the periods of cyanide injection during physiologically controlled ventilation were accompanied by an increased lactic acid content and increased oxygenation of the blood, the decreased hydrogen ion concentration is attributed primarily to the increased ventilation.

During the first part of the period of cyanide injection the total energy production (oxidative plus non-oxidative) was greater than the basal energy production. This gave way to a decreased production as oxidations were more markedly impaired. Subnormal dissipation of energy continued during recovery despite the tendency towards improved oxidations. In every case the combined period of administration and recovery showed a negative energy balance as compared with the basal rate. This negative balance coupled with the superbasal requirements for the extra work required to move the air, of warming the extra air, of saturation of the extra air with water vapor, etc., establishes a large deficit in required dissipation of energy.

The fact that a given augmentation of ventilation produced by cyanide administration is associated with a greater deficit of energy than a similar augmentation of ventilation produced by lowered alveolar oxygen suggests why cyanide administration is more harmful.

The intravenous administration of methylene blue during recovery from cyanide injection markedly accelerated the chemical changes usually associated with recovery.

Despite a sudden augmentation in the formation of carbon dioxide and in the partial pressure of carbon dioxide of the expired air there was a decrease in the hydrogen ion concentration of the arterial blood. This

decrease in hydrogen ion concentration was associated with a sharp decrease in blood lactic acid and an increase in the carbon dioxide capacity of the blood and a relative retention of carbon dioxide as indicated by the drop in the respiratory quotient to subnormal levels.

Despite the sudden increase in oxidations, with its consequent increased formation of carbon dioxide, ventilation remained virtually unchanged. This finding agrees with those of Eddy (1931) showing that methylene blue may increase or decrease the magnitude of pulmonary ventilation depending on the rate of oxidations obtaining at the time of injection.

The finding is also in agreement with the observation of Miss Pelecovich (1932) that the relation of pulmonary ventilation to the degree of impairment of oxidation is variable and uncertain.

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